## Add to the Body of Knowledge about Normal and Abnormal Biological Functions and Behavior

**IN THIS SECTION:**

SCIENCE ADVANCES | STORIES OF DISCOVERY

### SCIENCE ADVANCES

*FY 2002 NIH GPRA Research Program Outcomes*

Shape of a Brain Structure Predicts Behavior Problems in Adults Exposed to Alcohol as Fetuses
Even Moderate Drinking During Pregnancy May Be Risk to Offspring
Gene Regulates Two Behaviors Associated with Alcoholism
Brains of Alcoholics Reorganize to Perform Cognitive Tasks
Increased Use of Cigarettes, Alcohol, and Marijuana Among Manhattan Residents Following September 11th Attacks
Binge Use of Ecstasy is Toxic to the Heart
Although Brain Structure May Have capacity to Recover from Methamphetamine with Protracted Abstinence; Brain Function May Not
Prenatal Exposure to Cocaine Can Result in Significant Deficits in Mental Skills of Toddlers
Social Factors Can Change Neurobiology and Affect Vulnerability to Drug-Taking
Mutant Gene Associated with Drug Abuse
Prenatal Exposure to Methamphetamine Enhances the Toxic Effects Of Methamphetamine in Adult Male Rats
Understanding Addiction at the Molecular Level
3-D Visualization of Gene Expression in the Brain
Anthrax Toxin Structure Solved
Stop Cell Death, Help Treat Sepsis?
Studies of Iron-Pumping Bacteria May Lead to New Antibiotics
Bacteria Study Sheds Light on Cell Communication
A Protein to Tie up Loose Ends
A New Way to Relax – At the Molecular Level
Cells on the Move
Gene Silencer Also Controls Development
Cells That Live and Let Die
Anesthesia Dissected
Surprise Weapon to Treat Unwanted Angiogenesis
Anthrax Toxin Structure Solved
Gene-Environment Interaction Associated with Risk in Maltreated Children
Complex Trait Analysis of Transcriptional Networks
Mind Mapping: Cortical Cartography Takes on New Latitude
Mouse Model Provides New Insight into Memory Storage
Building an Animal Model of HIV Infection
Olfactory Neurons as a "Window" on the Etiology of Schizophrenia
Narrowing the Window of Fetal Vulnerability for Adult Schizophrenia
Face Processing in Autism Engages Unusual Neural Circuitry
Understanding the Molecular Basis of Antipsychotic Drug-Induced Weight Gain in Schizophrenia
Evaluating Threat in Social Situations: The Role of the Amygdala
Brain Circuitry Underlying Addiction, Obsessive-Compulsive Disorder
Novel Brain Mapping Reveals How Genes Affect Human Brain Structure
Amygdala Response to Fearful Faces
Pathophysiology of Auditory Hallucinations
Hypertension
Molecular Profiles Predict Post-Chemotherapy Survival Rates for B-cell Lymphoma Patients
Association of HLA and KIR Genes with AIDS Progression
Low Oxygen Levels Activate Kaposi's Sarcoma–Associated Herpesvirus (KSHV)
Mouse Model Provides Experimental Validation of a Critical Role for Childhood Sunburn in Melanoma Development
Annual Report to the Nation Helps Identify Needs in Cancer Research and Care

Inherited *BRCA2* Mutations Increase Risk for Pancreatic Cancer
Exploring the Tumor Microenvironments of Pancreatic and Breast Cancers
Scientists Identify Genetic Variations that may be Associated with Prostate Cancer
Gene Expression Analysis of Human Chondrocytes
Cartilage Injury and Intercellular Signaling for Cell Death: Implications for OA Progression
Differences in Damage Caused by Systemic Lupus Erythematosus among Hispanics, African-Americans, and Caucasians
Insulin Resistance in Myotonic Dystrophy
Abnormal Accumulation of Gene Messages in Myotonic Dystrophy
The Scientific Basis for the Use of Electrical Current to Heal Fractures
Understanding the Molecular Basis of the Hair Cycle and Hair Loss
Function of the Ichthyosis-Related Molecule Filaggrin in the Skin
Understanding the Molecular Basis for Lethal Versus Non Lethal Types of Epidermolysis Bullosa
Understanding Desmosome Structure and Function
Understanding the Basic Biology of Keloids
The Use of Large Scale Gene Expression Studies in Investigating Psoriasis
Molecular Basis and Animal Model System of Atopic Dermatitis
Children with Juvenile Rheumatoid Arthritis do not Have Permanent Knee Damage Early in the Disease
Molecular Basis for Differences between Human Joints
Heart Disease and Osteoporosis May Be Related Through a Common Mechanism
Rheumatoid Arthritis Patients at Increased Risk for Cardiovascular Events
Pattern of Inheritance of Familial Keloids Identified
New Insights Regarding Cardiovascular Disease and Osteoporosis in Women with Systemic Lupus Erythematosus
Differences Found in Clinical Manifestations of Systemic Lupus Erythematosus in Caucasians in Rochester, Minnesota, when Compared to Chinese in Singapore
Progressive Muscle Weakening Caused by Damaged DNA Synthesis Enzyme
Hormonal and Reproductive Risk Factors for Systemic Lupus Erythematosus
Anthrax Invades and Evades the Immune System to Cause Widespread Infection
Receptor Variant that Confers Enhanced Immune Function is a Marker for Atherosclerosis
Deafness, Retardation, and Attention Deficit Disorder – New Insight
Cigarette Smoke and High Cholesterol Increase Mitochondrial Damage in Cardiovascular Tissues
Genetic Subgroups Associated with Blood Pressure Response to the DASH Diet
New Gene Discovered That is Associated with Blood Triglyceride Levels
Evidence of Angiogenesis in Primary Pulmonary Hypertension
White Blood Cells Play an Important Role in Crises Caused by Red Blood Cell Sickling
New Evidence of Cardiac Stem Cells
Researchers Offer Alternative Explanation for Adult Stem Cells' Apparent Plasticity
Scientists Have Located Genes in Mice that Play a Role in Heart Failure Survival
New Link Found Between Viral Infection and Asthma
Study Finds Dramatic Decline in Physical Activity Among Black and White Girls During Adolescence
Stress Linked to Asthma Attacks
Sarcoidosis Study Finds Factors Influencing Susceptibility and Location
Gene Transfer Provides Long-Term Protection from Heart Damage in Rats
New Form of Aspirin Blocks Restenosis in Rats

Strategies for Protecting the Brain During Heart Surgery Hold Promise for Children
Cardiac Hypertrophy May Not be Necessary for Preserving Heart Function in Heart Failure Patients
The Role of Stress in the Deregulation of Blood Clotting May Explain its Role in Cardiovascular Disease
Can the Same Strategy Prevent Two Different Types of Vascular Disease?
Failure of AIDS Vaccine in Rhesus Monkey Provides Key Information for Better Vaccine Design
Researchers Discover How Anthrax's Killer Toxin Enters Cells
HIV Selectively Suppresses Anti-HIV Defense Cells
Researchers Identify Novel Asthma Susceptibility Genes
A Single Gene Change Aided the Emergence of Bubonic Plague
New Findings Provide Clues for Designing an Effective HIV/AIDS Vaccine
Cholera Bacteria Become More Virulent by Passing Through the Human Intestines
Enhanced Induction of Antibody Response to an HIV Vaccine
Scientists Discover New Influenza Virus Protein that Causes Cell Death
Clues into How the Immune System Fights Tuberculosis
Men and Women Respond Differently to Gonorrheal Infections
Viruses Help Strep Bacteria Become More Dangerous
Inflammation Control is Mediated by Adenosine Level
Older People Achieve Their Goal of Dying at Home
Role of Telomeres in Cellular Senescence
A New Mouse Model of Accelerated Aging Provides Insights Into the Aging Process
A Tale of Two Proteins
Prions, Misshapen Proteins, and Out-Of-Shape Brains
Adult Neural Stem Cells Make Functional Neurons
Neurons Know Where We're Going
Nitric Oxide Controls the Strength of the Heart Beat
Diabetes, ApoE_4 and the Risk for Alzheimer's Disease
Isolation of Neuron-Restricted Precursor Cells from Human Embryonic Stem Cells
Age Does Not Influence the Response to Resistive Strength Training
Uncovering the Secrets of Longevity
Regulation of a Critical Cell Cycle Checkpoint by the Breast Cancer Gene *BRCA1*
Hereditary Form of Prostate Cancer Linked to Gene on Chromosome 1
Low Vitamin C Levels May Be Linked to Massive Brain Bleeding and Lung Failure in Premature Newborns
Genetic Defect Responsible for Brain Disorder Among Amish Babies
Autoimmune Link in Juvenile Batten Disease
Progress in Understanding the Molecular and Genetic Basis of Inherited Epilepsy
Understanding How Inherited Defects Cause Facioscapulohumeral Muscular Dystrophy
Minocycline Delays Onset and Slows Progression of ALS in Mice
Delivering Therapy to the Brain with Molecular Trojan Horses
Watching Nerve Cells and Molecules in Living Animals
Animal Studies Yield Progress Toward Repairing Injured Spinal Cord
Genetic Analysis of Childhood Brain Tumors Improves Diagnosis, Predicts Survival, and Provides Insights Toward Developing More Effective Therapies
Microarray Analysis Yields New Targets for Drugs in Multiple Sclerosis
Cystamine Prolongs Survival and Decreases Abnormal Movements in an Animal Model of Huntington's Disease

Shedding Light on Biological Clocks
Protein Integrity May Be Key to Lens Transparency
A Gene Expressed in Brain and Retina is Associated with Glaucoma
Regeneration: A Developmental Switch
How are Sensory Representations Mapped in the Brain?
The Process of Light Adaptation Involves the Physical Movement of Proteins
Molecules that Mediate Ocular Inflammation ("Uveitis") Are Identified
Motivational Control of Behavior
An Unexpected Neuronal Specialization for Depth Perception
Identifying the Genetics of a Multiple Tumor Syndrome
New Mouse Model Mimics Rett Syndrome Features
Carriers of Fragile X Show Distinctive Characteristics
Harmless Virus Might Slow AIDS Progression
New Insights into Immune System Function
A Large Number of Human Genes are Found to Contain Transposable Elements
Vasectomy Does Not Increase Prostate Cancer Risk
Neuroimaging Useful in Understanding Dyslexia
A Possible Gene for Childhood Language Disorders
Rapid Renewal of Auditory Sensory Stereocilia Aid Recovery to Hearing Loss
A Sound Transduction Motor Protein Facilitates the Speed of Sound
Discovery of an Amino Acid Taste Receptor
Signaling Pathway Regulates Pillar Cell Development in the Inner Ear
Bacterial Biofilms Make Ear Infections Tough to Overcome
You Can Teach an Old Owl New Tricks: Plasticity in the Adult Brain
Loss of Sex Discrimination and Male-Male Aggression
A Critical Period for American Sign Language Processing
Do Stutterers Have Different Brains?
Study Suggests Link Between Periodontal Disease and Heart Disease
Two Newly Identified Genes Offer Broad Insights into Causes of Cleft Lip and Palate
Gene Identified as Causing Ectodermal Dysplasia Syndrome
Fundamental Discovery into Control of Motor Impulses
Studying Biology in its Natural Three Dimenional State
New Taste Receptor Identified
Genome of Oral Pathogen Fully Sequenced
Gene Identified as Causing Inherited Gingival Overgrowth
Advance in Understanding Biology of Pain

**STORIES OF DISCOVERY**

Exploring the Promise of Embryonic Stem Cells
Alcoholic Liver Disease
Harnessing Apoptosis to Destroy Cancer Cells
Lysosomal Storage Diseases
How The Brain Tells The Eye Where To Go
The Search for the Master Reproductive Hormone
Evolving Microbes

## Mutations Help Zero in on Zebrafish Genes

*Background*: The zebrafish is a superb model organism for studying genes of early vertebrate development. The small size and fecundity of adult fish, and the transparency and rapid external development of its embryo, make the species ideal for this purpose. To study the organism's genetic blueprint, researchers can disable genes, observe what happens, and discover the roles the genes play in development.

*Advance*: In previous large experiments, called screens, developmental biologists mutated zebrafish with a chemical called ethylnitrosourea, which efficiently creates thousands of deformed fish. However, pinpointing a single mutated gene requires breeding hundreds of fish and many months of detective work. Scientists at the Massachusetts Institute of Technology (MIT) tried another approach by infecting thousands of early zebrafish embryos with a genetically engineered mouse retrovirus that randomly disrupts genes. With this approach, termed insertional mutagenesis, retrovirally mutated genes can be tentatively identified in as little as two weeks. As part of a larger project, the MIT researchers produced 75 insertional mutants with abnormalities and identified each causative gene. Each of the 75 genes has at least some similarity to a known human gene or is clearly related to genes in humans. The DNA sequences can be used to assign probable biochemical functions to some of the genes, which encode a diverse array of proteins. Notably, the types of genes the researchers have identified produce a wide range of proteins, including many without known biological or biochemical functions.

*Implications*: The findings represent a major step in identifying the complete array of genes required for vertebrate development. Such studies aid understanding of how basic cellular processes influence the earliest stages of development.

Golling G, Amsterdam A, Sun Z, Antonelli M, Maldonado E, Chen W, Burgess S, Haldi M, Artzt K, Farrington S, Lin S, Nissen RM, and Hopkins N: Insertional mutagenesis in zebrafish rapidly identifies genes essential for early vertebrate development. Nat Gen 31: 135-140, 2002.

## Cancer Cells Spread via Lymphatic Vessels in Tumor Margins

*Background:* Cancer cells metastasize, or spread, to other areas of the body via blood vessels and lymph vessels, or lymphatics. Much is known about the growth of blood vessels in cancer and their role in metastasis, while little is known about the growth of lymphatics in cancer and their role in metastasis. The lymphatic system might be involved in other areas of health as well. Because lymphatics are hard to see, surgeons do not currently connect them during organ transplantation. Improvements in this area may lead to more successful transplantations. Also, areas of the heart that are well-drained by lymphatics are healthier, less fatty, and less prone to atherosclerosis. Finally, people who suffer from lymphedema, in which fluid accumulates in intercellular spaces due to lymphatics obstruction, would benefit if scientists were able to grow new lymphatics.

*Advance:* An investigator at the Maine Medical Center Research Institute's Center of Biomedical Research Excellence in Angiogenesis was part of a team that investigated whether intratumor lymphatics are functional. The researchers created tumor cell lines overexpressing a chemical known to stimulate the growth of lymphatics and implanted the cells in the hind limb of immunodeficient mice. Examination of the resulting tumors identified functional lymphatics in the margins of the tumors but not within the tumors. The scientists concluded that metastasis occurs via lymphatics in tumor margins and this area should be targeted in cancer treatment.

*Implications:* This discovery will be important in two research areas. First, it provides insight into the difference between the growth of lymphatics and the growth of blood vessels. Second, it provides insight into the importance of lymphatics in metastasis. Not only will this discovery affect the development of cancer treatments, it may also be important in the field of regenerative medicine. Scientists may eventually be able to engineer stem cells, which have some growth characteristics similar to those of cancer cells, to regenerate new organs. These organs will need lymphatics to remove waste products, among other functions. Therefore, knowledge of how lymphatics develop in tumors may have relevance for how they will develop in regenerated organs.

Padera TP, Kadambi A, di Tomaso E, Mouta-Carreira C, et al.: Lymphatic metastasis in the absence of functional intratumor lymphatics. Science 296: 1883-1886, 2002.

## Structures and Mechanisms of Ion Channels

*Background:* Inside the body, charged particles of potassium, chloride, and calcium perform a variety of vital functions. They enable nerve cells to convey signals, the heart to pump blood, and muscles to move the body. To perform these essential tasks, however, charged particles – or ions – must pass through cell membranes that are hostile to their electrical charges. Therefore, cell membranes contain specialized passageways, known as ion channels, that not only welcome charged particles but also are selective for particular ions. Because the channels are crucial to so many life functions, scientists have long sought to understand how ion channels operate. In particular, researchers have puzzled over the channel's ability to discriminate among charged particles and permit the transit of only one particular type of ion in response to metabolic cues.

*Advance:* As is often true in biology, the three-dimensional structure of an object holds the key to its functions. The laboratories of Drs. Roderick MacKinnon and Brian T. Chait at Rockefeller University have collaborated to solve the structures of several ion channels at an unprecedented level of detail. Aided by shared biomedical technology resources funded by NIH, the scientists used mass spectrometry and synchrotron radiation/x-ray crystallography to uncover the structure of gated potassium channels from bacteria. Their model of the channel demonstrates the mechanisms by which the channel selects for positively charged potassium ions and opens in response to the binding of intracellular calcium ions. By comparing the structures of open and closed channels, these scientists discovered that calcium binding changes the orientation of the proteins that make up the channel, allowing potassium ions to pass through.

In related investigations, Dr. MacKinnon and his colleagues have also deciphered the three-dimensional structure of a bacterial chloride channel, which provides the first glimpse of the architecture of a negative ion channel. The scientists were able to identify the structural elements that give the channel its specificity by recognizing and binding to chloride ions as they pass through the pore.

*Implications:* Groundbreaking studies like these provide biological researchers with insight into how ion channels function. Knowledge of the structures and functions of ion channels may offer clues to treating the many disorders caused or exacerbated by defective ion channels, including cystic fibrosis, kidney disease, and heart disorders.

Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R: X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature 415: 287-294, 2002.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R: The open pore conformation of potassium channels. Nature 417: 523-526, 2002.

Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R: Crystal structure and mechanism of a calcium-gated potassium channel. Nature 417: 515-522, 2002.

## Insulin Treatment Fails to Prevent Diabetes Mellitus

*Background*: Type 1 diabetes mellitus occurs in genetically predisposed persons as a result of the immune-mediated destruction of pancreatic islet beta cells that secrete insulin. Studies in laboratory mice have indicated that insulin treatment can prevent the onset of diabetes, and pilot studies have suggested that such treatment may also delay diabetes onset in humans. Because of these studies, many physicians have begun to administer insulin to persons who are at high risk for diabetes. To determine whether such treatment is of benefit, a large randomized, controlled clinical trial called the Diabetes Prevention Trial-Type 1 Diabetes was conducted. In this study, insulin was administered to relatives of type 1 diabetics, who are known to have a greater risk of developing diabetes than the general population.

*Advance*: Of 84,228 first- and second-degree relatives of patients with type 1 diabetes, 372 were determined to have more than a 50 percent risk of developing diabetes within the next five years. Of these, 339 agreed to be randomly assigned to undergo either close observation or an intervention consisting of twice-daily subcutaneous insulin injections plus annual four-day continuous intravenous insulin infusions. Median follow-up was 3.7 years. Diabetes was diagnosed in 69 subjects in the intervention group and 70 subjects in the observation group, suggesting that insulin therapy did not alter the relative risk of developing diabetes.

*Implications*: The outcome of this large study contrasts starkly with the results in mice and the results of pilot studies in humans. An important lesson is that clinical practice should not be altered solely on the basis of small pilot studies. Well-designed, randomized, controlled clinical trials are essential before guidelines for clinical practice are issued or public health practices are changed.

Diabetes Prevention Trial – Type 1 Diabetes Study Group: Effects of insulin in relatives of patients with type 1 diabetes mellitus. N Engl J Med 346(22): 1685-1691, 2002.

## PET Neuroimaging Tracks Cocaine's Effects on the Brain

*Background*: In recent years, scientists have used a variety of functional neuroimaging techniques to characterize the long-term effects of cocaine use on the human brain. These clinical studies have shown that chronic cocaine users have reduced blood flow in the prefrontal cortex, a brain region that influences critical thinking, attention span, and judgment. Unfortunately, the high incidence of multiple-drug use among human cocaine addicts complicates the clinical study of cocaine's specific effects on brain function. Studies of nonhuman primates, however, offer the experimental control necessary to characterize the acute effects of cocaine exposure.

*Advance*: Investigators at the Yerkes National Primate Research Center used neuroimaging techniques to determine how cocaine affects brain blood flow in conscious rhesus macaques. The brain images produced by positron emission tomography (PET) revealed that, within five minutes of cocaine injection, blood flow increased significantly in the dorsolateral regions of the prefontal cortex, indicating an activation of these brain areas. In a follow-up study, scientists administered the drug alaproclate – a selective serotonin reuptake inhibitor (SSRI) – to monkeys half an hour before cocaine administration. Pretreatment with alaproclate appeared to block cocaine's effects on the brain, since PET images did not indicate an upsurge in brain blood flow after cocaine exposure. The results document a distinct pattern of cocaine-induced brain activation that can be blocked with SSRIs.

*Implications*: With the ability to noninvasively monitor brain activation in conscious nonhuman primates, scientists can assess the effects of varying doses of cocaine and other drugs on the brain. This will enhance their ability to identify patterns of brain activation that occur with addiction and to evaluate potential therapies for blocking or reversing the effects of cocaine use or addiction.

Leonard L, Howell JM, Hoffman JR, Votaw AM, Landrum KM, Wilcox KP, Lindsey K: Cocaine-induced brain activation determined by positron emission tomography neuroimaging in conscious rhesus monkeys. Psychopharmacology 159: 154-160, 2002.

## "Glue" Proteins Maintain Nerve Cell Connections in the Worm

*Background:* During development, nerve cells, or neurons, extend processes called axons along specific routes to target cells, such as other neurons or muscle cells. Scientists studying neuronal development have primarily focused on identifying factors that guide axonal growth while neuronal connections are being formed, while little attention has been paid to identifying factors that maintain these connections once they are formed. Because of its relatively simple nervous system, the roundworm *Caenorhabditis elegans* provides a useful experimental model for studying early nervous system development. In the posterior, or tail, section of the worm are several neurons that send axons into a nerve bundle called the ventral nerve cord. These neurons are designated with three letters, the first two being P (for posterior) and V (for ventral). The third letter denotes the sequence in which the neurons were originally discovered and named. One of these neurons, PVT, secretes a protein that guides the growth of axons during embryonic development of the ventral nerve cord.

*Advance*: Researchers from Columbia University and the NIH-funded Center for *C. elegans* Anatomy at the Albert Einstein College of Medicine discovered that PVT also secretes six antibody-like proteins during later developmental stages called the larval stages, as well as in adult animals. To determine PVT's role in these later stages, the investigators destroyed PVT during the first larval stage. By this time, motor neuron axons have already grown into the ventral nerve cord. In about a third of the worms, PVT deletion caused VNC axons to lose their connections and move to the opposite side of the cord. Similar results occurred in worm mutants lacking one of the six proteins. The scientists propose that this protein is one of the factors produced by PVT that maintain axons in their proper position, starting in larval stages and continuing into adulthood.

*Implications*: This is one of the first demonstrations that a specific gene product can influence the maintenance of axonal positions within an animal. Such mechanisms may be widely used in animals, including humans, to ensure the preservation of functional neuronal circuits. Disruption of these mechanisms may be factors in neurological disorders and normal aging. More generally, these studies demonstrate the utility of a well-characterized model organism such as *C. elegans* to study basic features of animal development.

Aurelio O, Hall DH, Hobert O: Immunoglobulin-domain proteins required for maintenance of ventral nerve cord organization. Science 295: 686-690, 2002.

## Three-Dimensional Mapping of Gene Expression in the Worm

*Background*: Whole-genome sequencing has enabled the discovery of large numbers of genes, although the functions of most are unknown. Scientists have developed high-throughput procedures for studying many genes in parallel and have determined which genes are expressed, or turned on, in various cells and tissues. However, researchers would like to have a comprehensive, global technique for simultaneously analyzing and uncovering clues to the functions of the many thousands of genes in an organism. The roundworm *Caenorhabditis elegans* is a useful creature on which to test such potential technologies. The worm's genome has been fully sequenced and about 19,000 genes identified, more than half of which are similar to human genes.

*Advance*: Researchers at Stanford University have developed a novel computer-based technique that generates three-dimensional (3-D) topographical maps of gene activity, allowing scientists to visualize functional patterns of gene expression. Using this new visualization tool, known as VxInsight, the researchers pooled data from 553 different experiments, performed in collaboration with 30 different laboratories, on gene activity in *C. elegans.* Based on these data, VxInsight generated 3-D images that looked like mountainous terrain, with each mountain representing a cluster of genes associated with a specific cellular activity or tissue. The height of each mountain corresponds to the number of genes in that cluster, and the breadth of the base denotes functional correlations between genes. Scientists can visually explore this gene-expression landscape to gain intuitive understanding of which genes work together to perform different activities.

*Implications*: Traditionally, gene function has been inferred from analysis of biochemistry and genetics, usually one gene at a time. But with advanced technologies like VxInsight, the functions of genes can now be inferred on a much larger scale, and patterns of activities can be identified. This type of functional analysis should be applicable to the genes of any organism, including humans and other mammals.

Kim SK, Lund J, Kiraly M, Duke, K, et al: A global expression map for *Caenorhabditis elegans*. Science 293: 2087-2092, 2001.

## Bacterial Genes Enable Reversible Genetic Control in Transgenic Mice

*Background:* Genetic manipulation of mice has provided useful information about the function of certain genes. However, the techniques used to date – knocking out genes or inserting new ones – have drawbacks. In some cases, the genetic manipulation kills many of the embryos, limiting the number of adult animals that can be produced. In other cases, the body reacts to an altered gene by activating otherwise silent genes, which can complicate the analysis of the function of the gene of interest. To overcome these problems, scientists have started introducing bacterial genes into mice that will allow tight, reversible control over certain mouse genes. One such bacterial gene system is the *lac* operon of the bacterium *Escherichia coli*, which controls the production of enzymes for metabolizing the sugar lactose. The regulatory components of this system consist of a gene for a protein called the *lac* repressor and its DNA-binding sequence, the *lac* operator. When lactose is absent, the *lac* repressor binds to the *lac* operator, preventing the activation of the genes for producing lactose-metabolizing enzymes. When lactose is added to the medium surrounding the bacterium, the lactose enters the cell, and binds to the *lac* repressor, causing it to detach from the *lac* operator. As a result, the enzyme genes become activated, the enzymes are produced, and lactose is metabolized.

*Advance*: Researchers at the University of Virginia have inserted *lac* repressor and *lac* operator genes into mice. However, instead of controlling the production of lactose-metabolizing enzymes, the genes control the production of tyrosinase, the enzyme that catalyzes the first step in the production of melanin, the dark pigment that provides color to the skin, hair, and eyes. In this system, the *lac* repressor binds to the *lac* operator, preventing tyrosinase production. As a result, no melanin is produced and the mice are albino. When a chemical similar to lactose, called IPTG, is given to the mice in their drinking water, IPTG binds to the *lac* repressor, causing it to detach from the *lac* operator. This activates the tyrosinase gene, which starts melanin production, and the mice transform from albino to colored.

*Implications*: This gene regulatory system allows scientists to turn mammalian genes on or off simply by adding a chemical to an animal's drinking water. This will greatly facilitate the study of gene function in health and disease.

Cronin CA, Gluba W, and Scrable H: The *lac* operator-repressor system is functional in the mouse. Genes and Development 15: 1506-1517, 2001.

## Knockout and Transgenic Pigs Offer a Window on Human Disease, Therapies

*Background:* Pigs are valuable animal models for studying aspects of cardiovascular disease and diabetes. Because their organs are similar in both size and physiology to human organs, the pig is also considered a potential organ source for cross-species transplantation, or xenotransplantation, into humans. Scientists have been seeking effective techniques for genetically manipulating pigs to produce specialized animals that might shed light on human disorders or be used for xenotransplantation. A primary obstacle to pig-to-human transplantation, however, is a sugar-based molecule that studs the surface of many pig cells but is not found in humans or most other primates. Because of this molecule, pig organs that are transplanted into primates are quickly recognized as foreign and rejected by the primate immune system.

*Advance:* Investigators from the University of Missouri and their collaborators recently reported two significant achievements in manipulating the pig genome to produce genetically engineered animals. In one study – one of the first successes in producing a transgenic pig – the scientists added the gene for the glowing green fluorescent protein (GFP) into the genome of pig egg cells, which were then fertilized, cultured, and transferred to recipient females. Two pigs born via this procedure had glowing tissues that expressed GPR. In the second study, the scientists used similar procedures to create the world's first "knockout" pigs, in which a single gene had been disabled. The knocked-out gene was one of a pair that produce the pig enzyme galactosyltransferase, which is needed to generate the notorious sugar-based, cell-surface molecule that triggers human rejection of pig transplants. Of seven piglets born with a single knockout of the galactosyltransferase gene, four have survived at least nine months.

*Implications:* These experiments represent initial, essential steps for developing genetically engineered pigs suitable for xenotransplantation. They also represent a significant tour-de-force for large-animal nuclear transfer, which thus far has not been particularly successful in swine. The transgenic techniques that produced the GFP-expressing pigs can now be adapted to insert other genes of particular interest into the pig genome. However, many issues must be addressed before knockout pigs could be used for xenotransplantation. These include deactivating the second copy of the gene galactosyltransferase gene, as well as resolving many questions about cross-species transplant rejection and potential transmission of endogenous viruses.

Lai L, et al: Production of alpha-1,3galactosyltransferase knockout pigs by nuclear trnasfer cloning. Science, 295:1089-1092 (2002).

Cabot RA, et al: Transgenic pigs produced using in vitro matured oocytes infected with a retroviral vector. Animal Biotechnology 12: 205-214, 2001.

## Mouse Mutations Lead to Degeneration of Purkinje Cells

*Background:* Mice with mutations that affect the nervous system can be studied to uncover the functions of specific cell types, both normal and abnormal. More than 25 years ago, researchers identified a naturally occurring mutant mouse marked by brain degeneration – especially loss of Purkinje cells in the cerebellum – during early adulthood. Dubbed *pcd* for *Purkinje cell degeneration,* this mutant mouse also exhibits loss of retinal photoreceptors, neurons in the olfactory bulb, and selected thalamic neurons; males are infertile because of abnormal spermatogenesis. Despite years of study, scientists had not pinpointed the gene responsible for *pcd* until 2002.

*Advance:* Researchers at St. Jude Children's Research Hospital and The Jackson Laboratory recently identified two additional variants, or alleles, of *pcd* mice that arose spontaneously in captive mouse colonies. Studies of the allelic *pcd* mice aided the identification of the causitive gene, designated Nna1, which was shown to be abnormal in all three variant mouse colonies. Nna1 is remarkably similar to a previously identified human gene and is believed to encode a nuclear protein associated with nerve regeneration.

*Implications:* Naturally occurring mutants like the *pcd* mice provide a valuable tool for investigating the molecular underpinnings of neuronal degeneration. Such studies may provide insight into the mechanisms of neurodegenerative diseases and male infertility.

Fernandez-Gonzalez A, LaSpada AR, Treadaway J, Higdon JC, Harris BS, Sidman RL, Morgan JI, Zuo J: Purkinje cell degeneration (pcd) phenotypes caused by mutations in the axotomy induced gene, Nna1. Science 295: 1904-1906, 2002.

## Heat-Induced Stress and Molecular Protection in Zebrafish

*Background:* From bacteria to humans, many organisms produce protective molecules known as heat shock proteins (HSPs) when under stress. By aiding repair of proteins damaged by environmental challenges, HSPs help to shield cells and tissues from injury and destruction. The genes that produce HSPs are activated by signaling molecules known as heat shock transcription factors (HSFs), which respond to a variety of physiological signals, including excess heat. Scientists are trying to tease apart the many molecular interactions that occur in response to stress and are searching for interspecies similarities in these protective activities.

*Advance*: To determine if the function of the heat shock transgenic factor HSF1 is conserved between zebrafish and higher organisms, zebrafish HSF1 expression was inhibited during development using morpholino technology. Morpholinos, antisense oligonuleotides that deactivate RNA sequences, are considered a promising new tool for studying gene function, control, and interactions between gene products. Morpholino oligonuleotides specific for the HSF1 sequence were injected in embryos of transgenic zebrafish carrying an *Hsp70-promoter-GFP* reporter construct. Results indicate that the morpholino-injected embryos exhibit a severe reduction in expression of the heat-induced HSP70-GFP reporter gene and an increase in cellular death at elevated temperatures.

*Implications*: The use of morpholinos with transgenic technology provides a powerful and effective tool for studying heat-induced cell death *in vivo* during zebrafish development. The heat shock transcription factor HSF1 is conserved in zebrafish and higher vertebrates.

Wang G, Huang H, Dai R, Lee K, Lin S, Mivechi NF: Suppression of HSF1 in zebrafish causes apoptosis. Genesis 30: 195-197, 2001.

## Nonhuman Primates Shed Light on Attention Deficit Hyperactivity Disorder

*Background:* Attention deficit hyperactivity disorder (ADHD) – a condition marked by excessive impulsivity, distractibility, and activity – is the most commonly diagnosed behavioral disorder of childhood, affecting an estimated 3-5 percent of school-age children, according to a 1999 U.S. Surgeon General Report. Despite recent advances in assessing, diagnosing, and treating ADHD, the underlying biochemical and genetic factors that contribute to the condition are poorly understood. At least one clinical study identified elevated levels of dopamine transporters in the brains of adults with ADHD; however, a good animal model is needed to further explore the pathogenesis of ADHD. A rat model for hyperactivity has been identified, but rodent and primate genes for the dopamine transporter (DAT) are too dissimilar to shed light on DAT's role in human ADHD.

*Advance:* Scientists at the New England National Primate Research Center have conducted a series of studies that point to the rhesus macaque as a suitable animal model for ADHD. The macaque has nearly 100 percent gene homology with human DAT and well over 95 percent gene homology for other cellular transporters and receptors in the human brain. Like most genes, DAT also contains untranslated regions, sometimes referred to as "junk" DNA. The researchers found that variability in these untranslated segments appears to influence levels of DAT protein in the primate brain. In addition, the scientists compared expression of two different DAT alleles in five “more hyperactive” and five “more sedate” macaques. Two genotypes were found to be suggestive, but not predictive, of hyperactive behavior. Sequence analysis revealed potential single nucleotide polymorphisms that may account for the diversity of DAT alleles between individuals, further refining genetic analysis capabilities.

*Implications:* Understanding DAT expression within the brain may be key to improving the diagnosis and treatment of ADHD. Because the genetic makeup of the rhesus macaque is remarkably similar to that of humans, the macaque appears to be an excellent animal model for studying ADHD behaviors, neurophysiology, and pathogenesis. Such studies may also aid understanding of additional dopamine-related human disorders, including Parkinson's disease, schizophrenia, and substance abuse.

Madras BK, Miller GM, Fischman AJ: The dopamine transporter: Relevance to attention deficit hyperactivity disorder. Behavioural Brain Research 130: 57-63, 2002.

Miller GM, Madras BK: Polymorphisms in the 3’-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. Molecular Psychiatry 7: 44-55, 2002.

Miller GM, De La Garza II R, Novak MA, Madras BK: Single nucleotide polymorphisms distinguish multiple dopamine transporter alleles in primates: Implications for association with attention deficit hyperactivity disorder and other neuropsychiatric disorders. Molecular Psychiatry 6: 50-58, 2001.

## Understanding the Molecular Basis of Fear and Anxiety Management

*Background*: Research advances over the past few decades have significantly improved our understanding of how the brain receives and translates information into chemical and electrical signals. However, little is known about how these signals are converted into uncomfortable emotional responses and memories, such as fear, anxiety, and trauma. Furthermore, scientists do not understand how these memories are minimized or eliminated. Individuals with anxiety disorders, such as post-traumatic stress disorder, are often unable to disassociate the fear-inducing stimulus from the original stressful incident. These individuals may have difficulty letting go of painful memories or learning new ways to cope with their anxiety.

*Advance*: With funding from NIH's Institutional Development Award Program, researchers at The Jackson Laboratory in Bar Harbor, Maine, and at the University of Vermont in Burlington have developed an animal model to study the molecular and neurological mechanisms that control anxiety. The investigations used mice that have a mutant gene known as *cdf,* which leads to abnormal-looking cells in specific regions of the brain. The mutant mice also display certain atypical behaviors, including a reduced startle response. Normal mice learn to associate a sound to a mild shock to the foot; even in the absence of the mild shock, normal mice are still startled in response to the sound only. However, the *cdf*-mutant mice do not learn the startle response, suggesting that the *cdf* gene might be involved in maintaining certain memories. The researchers further showed that the mutant mice fail to make a protein called alpha-N-catenin. However, when the ability to produce this protein was restored, the mice returned to normal. Their brains resume a normal appearance and their startle response matches that of normal mice.

*Implications*: This project is the first to evaluate systematically the molecular and neurological mechanisms associated with the learned reduction of fear. The study also provides a viable animal model for understanding how the memory of fears is minimized or eliminated. These findings may eventually lead to new and more effective treatments for individuals suffering from anxiety disorders.

Park C, Falls W, Finger JH, et al: Deletion in *Catna2*, encoding alpha-N-catenin, causes cerebellar and hippocampal lamination defects and impaired startle modulation. Nat Gen 31: 279-284, 2002.

## Genetic Variation in Essential Hypertension

*Background:* About 95 percent of all cases of high blood pressure are classified as "essential" hypertension, meaning there is no identifiable cause. Essential hypertension constitutes a major public health risk, contributing to stroke, heart attack, heart failure, and kidney failure. The condition appears to have a genetic component, although scientists have been unable to pinpoint a genetic cause. Researchers have, however, identified molecular processes in the kidney that may play a role in the pathogenesis of inherited essential hypertension. For instance, tubule cells in the kidneys produce dopamine, a chemical that helps to lower blood pressure by binding to cell-surface molecules known as $D_1$ receptors. This binding, in turn, leads to production of enzymes known as the G protein enzyme complex, which causes sodium excretion after increased sodium intake. Previous research has shown that disruption of the $D_1$ receptor causes hypertension in mice.

*Advance:* NIH-supported scientists at Meharry Medical College participated in a multi-center study that examined the molecular and genetic basis of essential hypertension. The researchers analyzed cultures of human renal tubule cells collected from patients with essential hypertension, as well as patients with normal blood pressure. The scientists identified three variants of a single gene that appear to be linked to hypertension. Biochemical analyses revealed that each of these three mutations causes the $D_1$ receptor to uncouple from its G protein enzyme complex in the renal tubule. Moreover, in a related animal experiment, mice expressing a variant gene (as opposed to the normal, wild-type gene) developed hypertension.

*Implications:* This research identified genetic variants in the human genome that may be predictive of the risk for essential hypertension. Moreover, a mechanism for defective $D_1$ receptor coupling was described that may explain the kidney's failure to properly excrete sodium in some cases of essential hypertension.

Felder RA, Sanda H, Xu J, et al: G protein-coupled receptor kinase 4 gene variants in human essential hypertension. Proc Nat Acad Sci 99(6): 3872-3877, 2002.

## Age Differences and Estrogen Effects on the Immune System

*Background:* The female and male sex hormones, estrogen and androgen, have been shown to suppress the formation of B lymphocytes, a type of white blood cell that originates in the bone marrow and fights infection by producing antibodies. When adult mice are treated with estrogen, B lymphocyte production declines, and when adult sex hormones are removed, B lymphocyte formation rises. However, scientists have been puzzled by the fact that fetal mice are able to generate B lymphocytes in utero, despite their exposure to high levels of maternal estrogen and fetal sex hormones. This raises the possibility that sex hormones may have differing effects on the adult and the fetal immune systems.

*Advance:* Scientists at the Oklahoma Medical Research Foundation in Oklahoma City developed methods for isolating and culturing rare bone marrow stem cells, which are precursors to B lymphocytes, in adult and fetal mice. The investigators found that adult, but not fetal, stem cells are sensitive to estrogen. In addition, blood-forming stem cells in the bone marrow do not acquire receptors, or molecular attachment points, for sex hormones until after birth. In newborn mice, androgen receptors begin to appear on the stem cells at least two weeks before estrogen receptors, which may explain why males and females have different incidences of some diseases.

*Implications:* People of any age may need boosting of their immune systems to fight infections or to regain immune protection following cancer radiation or chemotherapy, which kills bone marrow cells. The basic scientific information gleaned from this study may inform the development of therapies that target the immune system. This study also yields information about important differences between fetal and adult stem cells, which have implications for tissue regeneration of many kinds.

Igarashi H, Kouro T, Yokata T, Comp PC, Kincade PW: Age and stage dependency of estrogen receptor expression by lymphocyte precursors. Proc Nat Acad Sci 98(26): 15131-15136, 2001.

## Brain Repair Mechanisms in "Motheaten" Mice

*Background:* More than three decades ago, scientists identified a naturally occurring mutant mouse with many unusual characteristics. Notably, certain brain regions in these animals were so riddled with microscopic holes that they appeared to have been nibbled by moths. Dubbed motheaten mice, these mutants also overproduced certain kinds of white blood cells in the lungs and skin. More recently, scientists fingered the causative gene – a disabled form of the gene that encodes the protein tyrosine phosphatase SHP-1, which is primarily produced in cells of the immune system. SHP-1 is also made by some glial cells, which help generate the myelin insulation for nerves in the brain and spinal cord. SHP-1, in turn, contributes to the creation of star-shaped connective-tissue cells known as astrocytes, found in the central nervous system.

*Advance*: Researchers at the University of Montana in Missoula examined the role of SHP-1 in the development of the mouse central nervous system. The scientists found that "motheaten" brain regions had low numbers of astrocytes and glial cells and also lacked myelin. The scientists therefore concluded that SHP-1 must play an indispensible and dual role in the CNS, enabling normal development of connective and insulating tissues and triggering production of astrocytes and glial cells as part of the normal repair process.

*Implications:* This work provides an understanding of biochemical pathways that may contribute to treating brain or spinal cord injuries. Basic research studies such as this one also help establish the research tools, such as mutant mouse strains, needed to understand the complex connections between genetic mutations, biochemistry, altered biological functions, and disease states.

Wishcamper CA, Coffin JD, Lurie DI: Lack of the protein tyrosine phosphatase SHP-1 results in decreased numbers of glia with the motheaten (me/me) mouse brain. J Comp Neurol 441: 118-133, 2001.

## Gut Hormones That Influence Weight Regulation

*Background:* Long-term control of body weight is achieved through balancing food intake and physical activity. The "appetite control center" in the brain monitors an array of chemical signals generated by fat, muscle, and other tissues in order to assess energy needs and stores and modify appetite and physical activity accordingly – thereby ensuring energy balance. The gut plays a key role in the control of body weight not only by absorbing nutrients, but also by providing both hunger and satiety signals. Many of these signaling molecules are just now being identified.

*Advance:* Ghrelin is a hormone secreted by the stomach and upper intestine that stimulates appetite. Bloodstream levels of ghrelin cycle throughout the day, peaking just before a meal and declining to a pre-set baseline level soon after. Studies in both animals and normal human volunteers reinforce a role for this hormone as a signal to eat. Researchers recently found that, in response to weight loss of 17 percent, dieters' overall ghrelin levels increased by an average 24 percent – a compensatory response typically observed with signaling molecules involved in maintaining energy balance. Complementing this finding, these investigators discovered that ghrelin secretion is nearly non-existent in persons who have had Roux-en-Y gastric bypass (RGB) surgery, a treatment for morbid obesity in which the top of the stomach is surgically connected to the middle of the intestine. This observation is consistent with the decrease in appetite experienced by the majority of RGB surgery patients, and may explain their success in maintaining long-term weight loss. In related work, the group examined ghrelin levels in patients with Prader-Willi syndrome (PWS), a genetic disorder causing the most common form of human syndromic obesity. In contrast to persons with diet-induced obesity, who have what appears to be a compensatory drop in ghrelin levels, PWS patients have significantly elevated baseline levels of ghrelin, suggesting that derailed regulation of ghrelin secretion may contribute to weight gain in this disorder.

In contrast to ghrelin, the gut hormone $PYY_{3-36}$ appears to inhibit appetite. This hormone is secreted by the intestine in response to a meal. Researchers found that injecting $PYY_{3-36}$ to achieve levels similar to those after a meal had long-term effects on energy balance: it decreased total food consumption and reduced weight gain in rats, and inhibited appetite and reduced food consumption in humans. They further characterized the activity of the hormone in the brain pathways regulating appetite. They found that $PYY_{3-36}$ indirectly stimulates specific cells (POMC neurons) in the pathway that leads to appetite inhibition. Furthermore, mice lacking a putative brain cell receptor for $PYY_{3-36}$, known as Y2R, no longer showed appetite inhibition when injected with $PYY_{3-36}$. These results suggest a possible mechanism for the observed activity of $PYY_{3-36}$.

*Implications:* An estimated 64 percent of adult Americans are overweight or obese, a strong risk factor for heart disease and type 2 diabetes. These advances demonstrate a strong correlation between levels of two gut hormones, ghrelin and $PYY_{3-36}$, and weight regulation through appetite. Since appetite control is one of the greatest challenges for dieters, understanding the

activities of ghrelin and $PYY_{3-36}$ in the body may prove to be an important key for developing interventions to control weight or achieve and sustain weight loss.

Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ: Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 346(21): 1623-1630, 2002.

Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, Weigle DS: Elevated plasma ghrelin levels in Prader Willi syndrome. Nat Med 8(7): 643-644, 2002.

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR: Gut hormone $PYY_{3-36}$ physiologically inhibits food intake. Nature 418: 650-654, 2002.

## Multipotent Adult Progenitor Cells Derived from Bone Marrow

*Background:* With the hope of one day replacing diseased or damaged tissue with normal cells, scientists are exploring stem cells and "progenitor" cells as possible sources of cells for therapeutic use. Stem cells can develop, or "differentiate," into the specialized cell types of the body, such as liver cells and blood cells. Embryonic stem cells can differentiate into all of the body's specialized cell types. While adult stem and progenitor cells are known to differentiate into the cell types of the tissue in which they reside, there are tantalizing reports that they may also be able to differentiate into other cell types. This past year, scientists identified adult bone marrow progenitor cells that have many of the properties of stem cells: they can differentiate into an extraordinarily broad repertoire of specialized cell types. These cells are referred to as multipotent adult progenitor cells, or MAPCs.

*Advance:* The scientists first identified MAPCs in human bone marrow, demonstrating that these cells could be coaxed to differentiate into a variety of specialized cell types, including bone cells, fat cells, blood vessel cell types, and nervous system cells. Adapting the techniques they developed for human cells, the scientists then extricated MAPCs from the bone marrow of mice and rats to facilitate later experimentation in animal models. To explore further the differentiation potential of MAPCs, the scientists investigated whether these cells could morph into yet another cell type – liver cells. They isolated MAPCs from the bone marrow of human donors and from mice and rats, and tried growing the MAPCs in different ways to coax them to differentiate into liver cells. They then screened the differentiated cells for liver-specific biological markers and subjected the cells to a battery of functional assays; the tests revealed that the MAPCs could differentiate to acquire a distinct set of liver-cell traits, adding another cell type to their repertoire. Next, the scientists demonstrated that these different specialized cell types did in fact arise from a single "multipotent" MAPC, and were not simply descendants of several different less-potent progenitor cells. By inserting special genetic tags into mouse MAPCs to uniquely mark the DNA of each MAPC and all cells derived from it, the scientists were able to trace the lineage of diverse specialized cell types back to the same original MAPC. Similar experiments with human MAPCs confirmed that they, too, are multipotent. The scientists also showed that both human and rodent MAPCs can grow and divide extensively in the laboratory, repeatedly doubling their numbers while maintaining their differentiation potential. Further, despite these generations of cell divisions, the MAPCs did not show molecular signs of "aging." Finally, the scientists investigated the differentiation potential of MAPCs in an animal. When put into mice, mouse MAPCs developed characteristics of specialized cells from many different tissues and organs. Additionally, while the MAPCs appeared to have differentiation potential rivaling that of embryonic stem cells, the MAPCs did not exhibit the unfortunate embryonic stem cell trait of forming tumors in the animals.

*Implications:* In theory, clinicians could eventually develop therapies that use MAPCs retrieved from a patient as back-up cells, ready to adapt and replace damaged tissue anywhere in the body. The use of a patient's own cells would also eliminate risks associated with transplants from donors. By bringing to light the remarkable differentiation potential of MAPCs, these studies

open new opportunities for stem and progenitor cell research and research on cell-based therapies.

Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418: 41-49, 2002.

Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu W-S, Verfaillie CM: Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest 109(10): 1291-1302, 2002.

## Stress Hormone May Determine Fat Deposition That Promotes Diabetes

*Background*: Obesity is one of the most common and fastest growing health problems in the U.S., with about 64 percent of the adult population considered overweight or obese. In the last two decades, the prevalence of obesity has more than doubled to 31 percent of the American adult population. Fifteen percent of American's youth are now overweight, with ominous implications for the nation's future health. Obesity is associated with a number of diseases, such as heart disease and stroke, as well as type 2 diabetes. If individuals take in more energy – in the form of calories from food – than they expend in exercise or metabolic processes, then obesity can result. The excess energy is most efficiently stored by the body as droplets of fat within specialized fat cells, called adipocytes, that develop and grow in different parts of the body. However, the best predictor of obesity-associated diseases such as diabetes is not the total body fat, but the amount of abdominal or "visceral" fat leading to the so-called "apple-shape."

*Advance*: Recent research indicated that the hormone cortisol – the "fight or flight" stress hormone – may play a key role in determining where fat is deposited in the body. Researchers were drawn to study the role of cortisol because individuals with a rare illness known as Cushing's Syndrome have too much cortisol in their blood and develop severe abdominal obesity, insulin resistance, high blood pressure, and abnormal blood lipid levels, and often become diabetic. However, in contrast to those with Cushing's Syndrome, most obese individuals have normal levels of cortisol. Normally present in low amounts, active cortisol can be regenerated inside cells from an inactive form by an enzyme, 11β hydroxysteroid dehydrogenase type 1 (11β HSD-1). Researchers have shown that activity of this enzyme is increased about four-fold in the fat cells of obese individuals. This finding led scientists to investigate the significance of this enzyme in a mouse model. Scientists genetically engineered mice to moderately overproduce the cortisol-regenerating enzyme in their fat cells and compared them to normal mice. Initially, both groups of mice gained weight at the same rate when fed low-fat diets. However, as the mice entered adulthood, the genetically-engineered mice ate more and gained more weight. By 15 weeks of age, they weighed 16 percent more and carried a much larger proportion of their body weight around their abdomens than the normal mice. In addition, the level of cortisol in their abdominal fat was 15 to 30 percent higher than in the normal mice. As the genetically-engineered mice grew older, they also developed metabolic complications similar to those seen in obese individuals, including insulin resistance and abnormal blood lipid levels.

*Implications*: There is evidence that one drug already in use to treat type 2 diabetes reduces visceral fat by repressing the activity of the enzyme that regenerates cortisol in fat cells. Strategies that repress activity of this enzyme, and hence reduce cortisol production, may therefore become an effective treatment of visceral obesity and its complications. Increased understanding of the biology of fat cells may ultimately lead to improved approaches to managing and preventing obesity.

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. Science 294: 2166-2170, 2001.

## Genetic Loci Linked to Inflammatory Bowel Disease in Mice

*Background:* The inflammatory bowel diseases (IBD) known as Crohn's disease and ulcerative colitis affect nearly one million Americans. Typical early symptoms of IBD include abdominal pain, fever, watery or bloody diarrhea and weight loss, which can progress to malnutrition and growth retardation, compromise employment and social activities, and increase risk of intestinal cancer. A distinguishing characteristic between Crohn's disease and ulcerative colitis is the location of the inflammation associated with them. The cause or causes of IBD are unknown. One of the theories about the development of IBD is that the patient's immune system reacts inappropriately to bacteria that normally reside in the gut. This reaction initiates a cascade of molecular events that results in inflammation in the gastrointestinal tract. IL-10 is an anti-inflammatory molecule naturally produced in the body that, along with many other molecules, regulates inflammation in IBD. Molecules such as IL-10 which might provide resistance and others which might increase the disease inflammation are the products of individual genes which can be considered "susceptibility" genes that are difficult to identify due to the complexity of the disease.

*Advance:* To search for genes that influence IBD severity, researchers used a state-of-the-art technique known as quantitative trait locus (QTL) mapping in mice to identify chromosomal regions (loci) in which such genes may be located. For the study, the researchers first selected two strains of IL-10-deficient mice that develop disease that has some similarities to human IBD. One strain of mice is highly susceptible to gut inflammation, while the other is relatively resistant. The scientists then cross-mated mice from each strain and analyzed their hybrid descendants, which carried chromosomal DNA from both original strains. By quantitating the severity of disease symptoms in the mice and correlating the symptoms with the inheritance of different chromosomal loci, the scientists identified loci that are linked to IBD severity in IL-10-deficient mice. The most significant of these is on chromosome 3. The version of this locus that was inherited from the highly IBD-susceptible strain exacerbated disease, affecting nearly all of the symptoms that were studied. Loci on other chromosomes, including, interestingly, versions of loci from the relatively IBD-resistant strain, also contributed to disease symptoms. Further experiments, in which various combinations of these loci were analyzed, showed that the genetic complexity of IBD arises not only from the multiple loci that control its severity, but also from the different types of effects caused by genetic interactions among these loci.

*Implications:* The complete sequence of the mouse genome is now being assembled, providing sequence information about genes in mice that are homologous to human genes; if the mouse gene(s) on chromosome 3 that contribute to IBD severity can be identified, then it may be possible to identify similar genes which cause disease susceptibility in humans. This study emphasizes the complexity of molecular events leading to IL-10 deficiency-induced IBD in mice. The results provide new knowledge about the genetic underpinnings of IBD, but also emphasize the difficulties to be overcome in finding therapies for patients.

Farmer MA, Sundberg JP, Bristol IJ, Churchill GA, Li R, Elson CO, Leiter EH: A major quantitative trait locus on chromosome 3 controls colitis severity in IL-10-deficient mice. Proc Natl Acad Sci 98(24): 13820-13825, 2001.

## Novel Approaches to the Therapy of Obesity

*Background:* Obesity has emerged as one of the greatest threats to human health and well-being, with the number of those affected continuing to escalate at an alarming rate. These individuals are at increased risk for coronary heart disease, diabetes, stroke, and some forms of cancer. Obesity occurs when the balance of food intake and energy expenditure is perturbed. Maintaining this balance depends on complicated interactions of many biological and behavioral factors, including genetic predisposition, food intake, and activity level. Although vigorous research has uncovered genes and metabolic pathways that contribute to obesity, a cure remains elusive. Thus, scientists continue to seek answers and look for novel approaches to find a means to treat and prevent obesity.

*Advance:* One research approach explored the potential for using a molecule that mimics insulin to control weight. Insulin is a hormone, produced in the pancreas, that interacts with receptors on brain cells to modulate energy balance. Through a set of experiments in animals, researchers showed that insulin injected into the brain reduces food intake and body weight. In contrast, insulin administered systemically has no effect on curbing obesity, and actually leads to weight gain. Similar experiments were carried out using small molecules that mimic insulin (insulin "mimetics"). When administered to the brain of rodents, an insulin mimetic had the same weight-reducing effect as insulin, but when an insulin mimetic was given orally to rodents, it also reduced obesity. The ability of a small molecule insulin mimetic to control weight when administered orally gives it a significant advantage over natural insulin.

Drugs that increase serotonin activity in the brain are frequently used to assist in weight loss, because they cause appetite-control centers in the brain to reduce food intake. In the mid 1990s, one such drug, fenfluramine, often given in combination with phentermine, was widely prescribed as a weight-loss therapy. Although it was prescribed to millions for weight loss because it decreases food intake, fenfluramine and a related drug, dexfenfluramine, were removed from the U.S. market by the FDA due to reports that they caused high blood pressure in the lungs and heart valve damage. Researchers studied the activity of dexfenfluramine (d-FEN) in rodents to determine how it reduces appetite. Their studies demonstrated that d-FEN causes one of the brain's appetite-control centers to release serotonin. The serotonin molecule binds to receptors on brain cells that release a hormone that causes a decrease in food consumption. Researchers may now be able to develop drugs that act along this pathway in a manner similar to d-FEN, but without producing the damaging side-effects that d-FEN and fenfluramine produce.

Scientists believe that when someone eats, a signal is sent to the brain. The brain responds through the sympathetic nervous system (SNS) to decrease appetite and increase energy expenditure in order to prevent excessive weight gain. The SNS mediates this response through the beta-adrenergic receptors located on targeted cells. This process is known as "diet-induced thermogenesis." To test this hypothesis, researchers generated mice without active beta-adrenergic receptors and compared them to normal mice. The test mice had a lower metabolic rate and were somewhat fatter than the normal mice when they were fed a typical chow diet and became massively obese when they were fed a high-fat diet. The researchers were also able to

demonstrate that the obesity that developed in mice without these receptors was the result of a lower metabolic rate and was not due to a decrease in activity or to an increase in food intake.

In other studies, scientists looked at a synthetic compound, C75, which is known to reduce appetite and body weight in mice. In an obese mouse model and normal control mice, a single dose of C75 caused reductions in food intake and body weight. However, when the mice were given lower doses of C75 over a longer time period, the normal mice initially reduced their food intake but became tolerant to C75 after the first day: their food intake returned to near normal and no additional weight was lost. In contrast, the obese mice continued to eat less and lose weight throughout the five-day trial. When researchers gave another group of control mice the same quantity of food as consumed by the C75-treated mice, they lost 25 to 50 percent less weight than the C75-treated mice. This discrepancy indicates that, in addition to suppressing appetite, C75 may stimulate an increase in the metabolic rate that accounts for the extra weight loss in the C75-treated mice.

*Implications:* Overweight and obesity affect approximately 64 percent of the U.S. adult population, with serious consequences for the health of the nation. As indicated by these studies, new opportunities to develop effective, innovative treatments for obesity are emerging from increased understanding of normal weight regulation, complemented by experimentation with novel compounds.

Kumar MV, Shimokawa T, Nagy TR, Lane MD: Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. Proc Natl Acad Sci 99(4): 1921-1925, 2002.

Air EL, Strowski MZ, Benoit SC, Conarello SL, Salituro GM, Guan X-M, Liu K, Woods SC, Zhang BB: Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nat Med 8(2): 179-183, 2002.

Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD and Elmquist JK: Activation of central melanocortin pathways by fenfluramine. Science 297: 609-611, 2002.

Bachman ES, Dhillon H, Zhang C-Y, Cinti S, Bianco AC, Kobilka BK, Lowell BB: BetaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297: 843-845, 2002.

## New Insights from Basic and Clinical Research on Hepatitis C

*Background:* Hepatitis C virus is one of the most common causes of liver disease in the U.S. While current drug treatments eliminate the virus in many people, they are totally ineffective or minimally effective in others, and it has not been clear whether a vaccine would be a useful alternative means for protecting against hepatitis C. Additionally, despite its proficiency at human infection, hepatitis C virus has been generally refractory to scientists' efforts to induce infections in small laboratory animals for use as models for research.

*Advance:* Scientists have now developed a novel mouse model of hepatitis C disease, and another research team recently investigated whether protection against viral persistence might be possible in humans. When hepatitis C virus infects humans, it inserts its genetic material into cells, so that the cells will produce viral proteins. Because the virus does not normally infect mice, a group of scientists came up with another way to put viral genes into these animals: they injected the genes into mouse eggs to generate transgenic mice that carried either the entire viral genome or a subset of the genes. The scientists had also linked the viral genes together with a segment of mouse regulatory DNA that turned on the genes in the liver. The mice accumulated excess fat in their livers and developed liver tumors, conditions commonly seen in hepatitis C infections in people. Given that the viral genes were innate to the transgenic mice and not introduced by infection, the potentially injurious inflammation that accompanies infection in humans was absent in these mice. The scientists concluded that viral proteins influence hepatitis C, although inflammation may also contribute to disease symptoms in human infection.

Vaccines for viral diseases are essentially mock infections with a weakened virus or virus fragment; they train the body to fight off later infections with a real virus. To see whether a vaccine approach might be useful for hepatitis C, scientists recently studied a group of people likely to have multiple exposures to this virus: users of injectable drugs. Some drug users had evidence of an earlier infection from which they had since recovered, while others had not been infected previously. Observations over the course of two years showed that those who had previous infections were over 10 times less likely to acquire a new persistent infection than those who had not been previously infected. In many of the people, prior infection seemed to confer protection against subsequent, persistent infections. Therefore, the scientists concluded that a vaccine approach to hepatitis C could be beneficial, because the serious liver diseases caused by this virus are associated with its persistence in the liver. This study also revealed that users of injectable drugs have an alarmingly high incidence of hepatitis C virus infection.

*Implications:* These studies provide insights into how hepatitis C wreaks havoc in the liver and how some level of protection against persistence of this virus in the body, and associated disease, might be achieved. This research will likely spur new efforts toward vaccine development.

Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, Okuda M, Gosert R, Xiao S-Y, Weinman SA, Lemon SM: Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology 122: 352-365, 2002.

Mehta SH, Cox A, Hoover DR, Wang XH, Mao Q, Ray S, Strathdee SA, Vlahov D, Thomas DL: Protection against persistence of hepatitis C. Lancet 359: 1478-1483, 2002.

**Hereditary Hemochromatosis: A Common Mutation May Not Lead to Common Disease**

*Background:* Hereditary hemochromatosis is a genetic disease that causes overabsorption of dietary iron. Because the body cannot excrete excess iron, its toxic accumulation can lead to a number of "secondary" diseases and complications, including diabetes, heart arrhythmias, and cirrhosis of the liver. Hereditary hemochromatosis is linked to mutations in the HFE gene, identified in 1996. Over 80 percent of hereditary hemochromatosis patients have a specific mutation in both copies of the HFE gene, a mutation called C282Y, which alters the HFE protein. However, there have been conflicting data about the actual risk of developing the disease when two copies of the C282Y mutation are present. Iron overload due to hereditary hemochromatosis can progress silently for many years, and the C282Y mutation is highly common – one in 200 to 500 Americans are C282Y homozygotes. Because hemochromatosis is easily treatable with phlebotomy (periodic bloodletting), but its associated diseases and complications are not so easily reversed, it is important to ascertain whether population screening for HFE mutations will effectively prevent disease.

*Advance:* To determine the predictive value of genotype (HFE C282Y) for phenotype (clinical disease), investigators screened 41,038 individuals for the C282Y mutation. They then assessed whether individuals with mutations were more likely to have symptoms or diseases associated with hereditary hemochromatosis than the control (non-mutant) group. Four genetic groupings were made: C282Y homozygotes, C282Y heterozygotes, C282Y/H63D "compound heterozygotes," and wild-type (non-HFE-mutant). 152 homozygotes and 616 compound heterozygotes were identified, of an average age of about 57 and with no gender bias. Iron burden was determined by two measures: saturation of the iron transporting protein, transferrin, and concentration of serum ferritin, an iron storage protein. By these measures, at least 75 percent of male and 40 percent of female C282Y homozygotes had significant iron burdens. However, the prevalence of most of the clinical conditions associated with hereditary hemochromatosis – including diabetes, arrhythmias, and impotence – was not statistically different between C282Y patients and wild-type controls. The only clinical symptoms occurring more frequently in C282Y homozygotes than wild-type was a history of hepatitis or other liver disorder, about a twofold increase. Only one individual homozygous for C282Y had the broad spectrum of clinical symptoms of hereditary hemochromatosis. From these results, the investigators estimate that less than one percent of C282Y homozygotes proceed to clinical disease.

*Implications:* Prevalence, intervention, and penetrance are the three most important factors in determining whether to engage in population screening for a genetic disease. The C282Y mutation in HFE is quite prevalent, and hereditary hemochromatosis is easily treatable. However, the results of this study suggest that the C282Y mutation alone is not an effective predictor of clinical disease. These findings will encourage investigators to seek secondary mutations or environmental factors – which may vary between populations – that influence the course of disease.

Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T: Pentrance of 845G-A(C282Y) *HFE* hereditary hemochromatosis mutation in the USA. Lancet 359: 211-218, 2002.

## Urinary Tract Infections: Emergence of a New Multi-Drug Resistant *E. coli* Strain

*Background:* Urinary tract infections (UTIs) caused by *E. coli* bacteria affect 11 percent of women annually. The widespread overuse of antibiotic drugs has led to increased incidence of multiple-drug resistance in *E. coli*, including those strains that can cause UTIs. Multiple-drug resistance severely limits the efficacy of existing antibiotics to treat infection.

*Advance:* Responding to a sharp increase in drug-resistant UTIs, researchers recently identified a new strain of *E. coli*, called clonal group A, in urine samples from women with UTIs in California, Michigan, and Minnesota. Clonal group A was responsible for both 10 percent of the total UTIs and 38 to 51 percent of the drug-resistant UTIs observed in the patient groups, a surprisingly high prevalence for a single strain. Importantly, the distinct geographic clusters of the patient groups studied suggests that there may be a common route of dissemination of clonal group A, possibly through food.

*Implications:* This advance stresses that molecular typing of the *E. coli* causing drug-resistant UTIs may provide important information about the origins and spread of these bacteria within communities, thus enhancing our opportunities to prevent further transmission of drug-resistant infections.

Manges AR, Johnson JR, Foxman B, O'Bryan TT, Fullerton KE, Riley LW: Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N Engl J Med 345(14): 1007-1013, 2001.

## Genes Involved in Polycystic Kidney Disease

*Background:* Polycystic kidney disease (PKD) affects 600,000 children and adults in the U.S. PKD is characterized by massive enlargement of the kidneys due to the presence of fluid-filled cysts and is the fourth leading cause of kidney failure. Ninety-five percent of the most common form of PKD is caused by mutation of either one of two genes, *PKD1* or *PKD2*. The proteins these genes encode, polycystin-1 and 2, respectively, can form a functional complex that may be involved in signal transduction in kidney epithelial cells. Recently, the gene causing a less common but more lethal form of PKD was identified: *PKHD1*. This gene encodes a novel protein, fibrocystin/polyductin, whose function is yet unknown. These discoveries have propelled research in model organisms to figure out how disruptions in PKD-related genes derail normal kidney tissue development or maintenance to cause cyst formation and progressive renal disease.

*Advance:* Two recent advances characterizing the expression of PKD-related genes in model organisms lend support to a role for ciliary dysfunction in the development of PKD. Cilia are hair-like projections found on certain cells. They can be used as "antennae" to sense and respond to changes in the extracellular environment. Polarized kidney epithelial cells – which line both normal kidney tubules and cysts – possess a single cilium per cell. In one advance, researchers identified and characterized the *cpk* gene, which is linked to PKD in one of several mouse models of this disease. The *cpk* gene is expressed primarily in mouse kidney and liver, and encodes cystin, a novel 145 amino acid protein. When an easily detectable version of cystin was expressed in polarized kidney epithelial cells grown in culture, it localized to the cilium in a manner similar to another PKD-related protein, polaris. Homologous versions of the *cpk* gene are present in other mammals, including humans, and in chickens, but not in the simple nematode worm *C. elegans*, suggesting that cystin is functionally specialized for the ciliated epithelium of higher vertebrates. In another advance, researchers studying the two *C. elegans* gene homologs of human *PKD1* and *PKD2 – lov-1* and *pkd-2*, respectively – found evidence through genetic studies that the two genes function in the same cellular pathway, similarly to human *PKD1* and *2*. *C. elegans* doesn't have kidneys, however. Instead, functional copies of both *lov-1* and *pkd-2* are necessary for proper male worm mating behavior. In protein localization studies, the researchers found that the *C. elegans* PKD-2 protein, like the LOV-1 protein, appears to be exclusively expressed in male-specific sensory neurons used in mating. These sensory neurons are ciliated; both LOV-1 and PKD-2 proteins are enriched in the cilia of these cells.

*Implications:* The role(s) of the cilium on kidney epithelial cells in normal development or maintenance of epithelial function or integrity is still under investigation. However, the results of these and other studies suggest that proper function of cilia-associated proteins may be necessary to prevent PKD. Further investigation of both cilia and PKD-related proteins is necessary. Furthermore, the specific observations of *lov-1* and *pkd-2* in *C. elegans* suggest that

this system may be useful as a basic model for understanding the function(s) of PKD1 and 2 in multi-cellular organisms.

Hou X, Mrug M, Yoder BK, Lefkowitz EJ, Kremmidiotis G, D'Eustachio P, Beier DR, Guay-Woodford LM: Cystin, a novel cilia-associated protein, is disrupted in the *cpk* mouse model of polycystic kidney disease. J Clin Invest 109(4): 533-540, 2002.

Barr MM, DeModena J, Braun D, Nguyen CQ, Hall DH, Sternberg PW: The *Caenorhabditis elegans* autosomal dominant polycystic kidney disease gene homologs *lov-1* and *pkd-2* act in the same pathway. Curr Biol 11: 1341-1346, 2001.

**Developing Strategies to Overcome Immune Rejection of Transplanted Cells and Organs**

*Background:* Critical to the success of any transplantation procedure is survival of the transplant in the recipient. However, the body's immune system is programmed to attack foreign material that enters the body – whether this material is infectious microbes, such as bacteria and viruses, or potentially life-saving organs. This problem has long plagued transplant patients, because agents used to suppress the immune system to prevent transplant rejection can also leave the patient more vulnerable to infection and other types of complications. Recently, scientists have obtained promising results in investigations of new drugs and drug combinations designed to modulate the immune system.

*Advance:* Scientists have developed a primate model of islet transplantation, a potential therapy for type 1 diabetes, based on an immunosuppressive strategy first developed in Edmonton, Canada for human islet transplantation. An animal model that is quite similar to humans will facilitate further evaluation and refinement of this still-experimental procedure for restoring insulin-producing capacity to patients whose own insulin-producing cells have been destroyed by an aberrant immune system. The scientists first induced diabetes in macaque monkeys, and then infused the monkeys with islets from other macaques. They also administered three immununosuppressive agents used in the human procedure: daclizumab, FK506 (tacrolimus), and rapamycin. In monkeys that maintained effective levels of these the agents in their blood, relief from the symptoms of diabetes was achieved. The immununosuppressive agents had subdued their immune systems into accepting islets from another animal, reproducing the effects seen in humans. Already, the scientists have gained important information from this primate model: they confirmed that the site of injection currently used to transplant islets into a recipient, the portal vein of the liver, is superior to another site.

Scientists are also investigating a new type of immunosuppressive agent to induce immune tolerance. This agent is a monoclonal antibody that binds to an important immune-cell protein called CD154. In recent years, the scientists demonstrated that anti-CD154 monoclonal antibodies are extraordinarily efficacious in preventing rejection of kidney transplants in monkeys. This past year, they put anti-CD154 antibodies to an even more rigorous test--skin transplantation. The scientists first selected pairs of donor and recipient rhesus monkeys that differed genetically in ways most likely to provoke immune rejection, to mimic a transplant between two entirely unrelated people. They then transplanted skin grafts onto the animals, and treated them with anti-CD154 antibodies. The anti-CD154 antibodies greatly enhanced the survival of the skin grafts in the transplant recipients.

*Implications:* The success of many transplantation procedures has been predicated on research into immune intervention to prevent transplant rejection. Immunosuppressive agents play an additional role in "autoimmune" diseases like type 1 diabetes. Since type 1 diabetes results from destruction of pancreatic islets by an aberrant immune system, immunosuppressive agents are needed not only to reduce transplant rejection, but also to help avert a recurrence of the immune

attack that caused the disease in the first place. The continued exploration of immunosuppressive agents in animals will likely translate into improved human health, not only through advances in immune modulation, but also through the development of animal models useful for optimizing other aspects of transplant procedures.

Hirshberg B, Montgomery S, Wysoki MG, Xu H, Tadaki D, Lee J, Hines K, Gaglia J, Patterson N, Leconte J, Hale D, Chang R, Kirk AD, Harlan DM: Pancreatic islet transplantation using the nonhuman primate (rhesus) model predicts that the portal vein is superior to the celiac artery as the islet infusion site. Diabetes 51: 2135-2140, 2002.

Harlan DM: Islet cell allotransplantation as a model system for a bioengineering approach to reparative medicine: immunological concerns. Ann N Y Acad Sci 961: 331-334, 2002.

Elster EA, Xu H, Tadaki DK, Montgomery S, Burkly LC, Berning JD, Baumgartner RE, Cruzata F, Marx R, Harlan DM, Kirk AD: Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primates. Transplantation 72(9): 1473-1478, 2001.

## Malaria in Pregnancy: Impact on HIV Transmission from Mother to Child

*Background*: Malaria and AIDS comprise two of the greatest threats to Africa's economic well-being and health and commonly occur simultaneously. Pregnant women with malaria are at greater risk for having children that are either low birth-weight or severely anemic and mother to infant transmission is one of the primary mechanisms by which HIV is transmitted. The interaction of malaria and HIV co-infection during pregnancy has significant consequences. For instance, studies show that mother-to-child transmission (MTCT) of HIV doubled during the malaria season in The Gambia. Despite these observations however, little is known about how malaria infection increases the severity of MTCT HIV transmission during pregnancy. In order to successfully enter into human immune cells, HIV must first bind to two proteins on the cell's surface, one being the CD4 protein and the other being the CCR5 protein. The CCR5 protein is thought to play an important role in mother-to-child HIV transmission. Infants that inherit a particular variation of this protein from both parents have a greater predisposition to being infected with HIV than those who inherit it from one or neither parent. Little is known about the presence of the CCR5 protein in the placenta, the site of MTCT. However, placentas of malaria-infected mothers often contain high numbers of immune cells (macrophages) that possess the CCR5 protein and, could therefore serve as reservoirs of HIV for transmission during pregnancy.

*Advance*: Scientists from Michigan and Malawi compared fetal and maternal immune cells with CCR5 in both malaria-infected and uninfected placentas. The CCR5 protein was detected only on maternal immune cells in placentas from malaria infected women. CCR5 protein was also detected on fetal immune cells. Overall, the placentas of malaria infected women contained three times as much CCR5 protein as placentas of women without malaria primarily due to increased numbers of maternal immune cells carrying CCR5.

*Implications*: Co-infection with malaria may increase HIV transmission from mother to child by increasing the number of potential maternal HIV reservoir immune cells in the placenta. Mother to child HIV transmission may be decreased by prophylactic anti-malarial drug treatment of pregnant women in areas with a high prevalence of both malaria and HIV.

Tkachuk A, Moorman A, Poore J, Rochford R, Chensue S, Mwapasa V, Meshnick S: Malaria enhances expression of cc chemokine receptor 5 on placental macrophages. Journal of Infectious Diseases 183: 967-972, 2001.

## Tuberculosis Mutant Reveals Basis of Pathology

*Background*: Approximately one-third of the world's population is infected with tuberculosis (TB) and each year more than two million people die of the disease. Most people infected with TB never develop symptoms of the disease although they remain persistently infected throughout life. There is controversy as to whether the clinical disease tuberculosis is primarily a dysfunctional immune reaction to the persisting microbe or whether the bacteria itself causes tissue damage. When Mycobacterium tuberculosis (MTB) infects a person, it spreads widely and multiplies slowly until immune mechanisms are activated to control its growth. In approximately 10 percent of those infected however, MTB multiplies uncontrolled in the lungs and perhaps elsewhere and triggers a host response that greatly contributes to tissue damage and results in the symptoms of active TB (including fever, persistent coughing, bloody sputum, and wasting) and eventually death. Scientists have little understanding of how TB transforms from a silent latent infection to an overt deadly disease.

*Advance*: Laboratory scientists in India and from Johns Hopkins have developed a strain of MTB containing a mutated version of the gene, *M. tuberculosis sigH*, that will help us to understand how the bacterium causes tissue damage. Strains with and without this mutant gene result in a distinct clinical effect in experimentally infected mice. In this model, the mutant strain infects, multiplies and survives in the lungs and spleen in the same manner as the standard TB mycobcterium, but does not stimulate inflammation, an immune response, or cause tissue damage as occurs with the original strain. Analysis of the mutant gene indicates that it directly regulates at least 31 genes and may modulate the expression of about 150 other genes. Many of these regulated genes appear to comprise a system that MTB uses to protect itself from heat, oxidation and other environmental stresses. Further research is required to understand the role of this system of regulated genes and how it functions in the regulation of the damaging immune response that results in clinical tuberculosis.

*Implications*: This study describes a novel pathway underlying the virulence of MTB. It suggests that strains containing genetic mutations that interfere with tissue damage could serve as vaccine candidates for tuberculosis. The mycobacterial environmental defense system identified in this study may also serve as a model for new drug targets that could be used to revert virulent to avirulent infections as a novel approach to chemotherapy of tuberculosis.

Kaushal D, Schroeder BG, Tyagi S, Yoshimatsu T, Scott C, Ko C, Carpenter L, Mehrotra J, Manage YC, Fleischmann RD, Bishai WR: Reduced immunopathology and mortality despite tissue persistence in a Mycobacterium tuberculosis mutant lacking alternative sigma factor, SigH. Proc Nat Acad Sci 99(12): 8330-8335, 2002.

## Organophosphorous Pesticide Exposure Increases the Frequency of Sperm Sex Null Aneuploidy

*Background*: Chromosomal aneuploidy is a genetic condition that results from an abnormal number of chromosomes in the developing embryo, this condition is caused by an abnormal number of chromosomes in either the ova or the sperm involved in the formation of the embryo. Although chromosomal aneuploidy is relatively rare, occurring in only 4 of every 1,000 live births, as many as 35 percent of spontaneous abortions (miscarriages) are aneuploid. Identifying factors that can reduce chromosomal aneuploidy represents an important step in reducing the risk of genetic mutations and the resultant spontaneous abortions. Previous studies have shown that an important proportion of embryo and newborn aneuploidy is of paternal origin. In addition, agricultural chemicals have been identified as potential causative agents for various birth defects and chromosomal abnormalities. More specifically, exposure to organophosphate pesticides (OP) has been associated with such abnormalities, including the chromosomal number in sperm. This study aimed to determine the relationship between short-term exposure to OP and aneuploidy in the sperm of agricultural workers.

*Advance*: Researchers from UCLA and Mexico assessed the frequency of sperm aneuploidy in chromosomes and its relationship with OP byproducts in urine samples from agricultural workers in the State of Durango, Mexico. Samples from nine men were obtained before and during the pesticide-spraying season to assess sperm aneuploidy in each season. The researchers found significant associations between the concentration of OP byproducts in urine samples and increased frequency of sperm aneuploidy. The association was more evident during the spraying season, and the most frequent aneuploidy was a missing sex chromosome. OP exposure interferes with normal sperm chromosome segregation and increases the risk for Turner's Syndrome or Kleinfelter's Syndrome that result from an absent X or Y chromosome. These conditions are more generically termed "Sperm Sex Null Aneuploidy".

*Implications:* Pesticides are known to cause abnormalities in biochemical pathways. The present work indicates that this potential harm can be caused in humans from a small amount of immediate exposure, such as during the spraying season and does not necessarily need a long time of exposure over years. This study is the first to show a correlation between OP exposure and Sperm Sex Null Aneuploidy. Because birth defects and spontaneous abortions are important and potentially damaging to the person affected and the family in a variety of ways (medical, social, financial), such information becomes very important. Further studies are required to assess the prevalence of spontaneous abortions, birth defects, and genetic syndromes in agricultural communities.

Recio R, Robbins WA, Borja-Aburto V, Moran-Martinez J, Froines JR, Hernandez RM, Cebrian ME: Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy. Environ Health Perspect 109(12): 1237-1240, 2001.

**Alcohol Researchers Discover First Genetic Link to Brain Waves**

*Background:* Electrical impulses travel from nerve cell to nerve cell in the brain. Networks of nerve cells work in concert to enable us to process information, ultimately influencing how we behave. Scientists can measure the frequencies of this electrical activity – "brain waves"– externally, with the electroencephalogram (EEG). Electrodes placed on the head detect oscillations in brain waves and map them out on paper. Each frequency shows a characteristic pattern on the EEG.

If the electrical stimuli in our brains were unregulated, chaos would result. Instead, the flow of electricity between nerve cells is governed by gates – molecular receptors for neurotransmitters, the chemical signals of the nervous system – that act something like traffic signals for cars. Some of these receptor systems speed up the electrical traffic of the brain, and some slow it down. Gaba-aminobutyric acid type A ($GABA_A$) is one of the receptor systems that slows it down.

Some substances, alcohol prominent among them, interfere with the speed and synchronization of electrical impulse transmission between nerve cells. Alcohol is known to interfere with both the $GABA_A$ receptor system and the beta frequency of the EEG. Underlying all of these events are genes, which produce the molecular systems that govern electrical impulse transmission. Until this finding, scientists had not linked any specific genes to any specific brain waves.

*Advance*: For the first time, researchers have linked a cluster of genes to a human EEG frequency. They found that a cluster of genes for $GABA_A$ receptors, on chromosome 4, are linked to the beta brain wave. Scientists of the Collaborative Study on the Genetics of Alcoholism used two types of statistical analyses, linkage and linkage disequilibrium, to reach this finding.

*Implications*: Certain variations in EEG frequencies correlate with certain pathological behaviors; people at risk of alcoholism, for example, tend to show increased activity in the beta frequency. Adding to previous findings, we now know that alcohol interferes with $GABA_A$ receptor function and disrupts impulse transmission and that beta brain waves are influenced by a cluster of genes on chromosome 4 that produce $GABA_A$ receptors. Together, these findings suggest that some of the genes that contribute to risk of alcoholism may reside in this cluster on chromosome 4.

The kind of finding described here will help scientists to identify genes that produce the neural machinery necessary for normal information processing and to identify biomarkers for diseases like alcoholism. Ultimately, this kind of information can help scientists track the root causes of pathology and identify points for therapeutic intervention.

Projesz B, Amasy L, Edenberg HJ, Kongming W, Chorlian DB, Foroud T, Goate A, Rice JP, O'Connor SJ, Rohrbaugh J, Kuperman S, Bauer LO, Crowe RR, Schuckitt MA, Hesselbrock V, Conneally MP, Tischfield JA, Li T-K, Reich T, Begleiter H: Linkage disequilibrium between the beta frequency of the human EEG and a GABA-A receptor gene locus. Proc Nat Acad Sci 99(6): 3729-3733, 2002.

## Environmental Factors Are a Major Contributor to Early Alcohol Use

*Background*: People who start using alcohol at young ages, in the childhood or early teen years, have a dramatically higher risk of becoming alcoholic at some point in life. This finding has raised a number of questions. Does early initiation of drinking *lead to* alcoholism? Or do certain characteristics predispose some people to engage in problem behaviors like early alcohol use *and* alcoholism, in addition to others? In either case, does the correlation between early alcohol use and alcoholism have a biological basis or an environmental basis, or both?

In this study, investigators followed a large group of twins from the time they were 11 or 12 years old until they were 14 years old. Studying twins is particularly valuable for alcohol researchers, because some twins – identical twins – have identical genetic compositions (fraternal twins don't). This better enables researchers to determine the effects of biological versus environmental factors. The twins themselves, their parents, and their teachers answered questions about the twins' home atmosphere, parental monitoring, pubertal development, social adjustment, and behavioral and emotional problems. Investigators then performed genetic and statistical analyses on the data they had gathered.

*Advance*: Environmental factors played a major role in determining whether or not twin siblings began using alcohol by age 14. Factors associated with early alcohol use included being female, having a twin of the opposite sex, early puberty, reduced monitoring by parents, less supportive home atmosphere, and behavior problems in school.

*Implications*: This study suggests that environmental factors play a prominent role in early alcohol use; however, the question of whether or not there is a cause-and-effect relationship between early drinking and subsequent alcoholism remains unanswered. Another study suggests that a common vulnerability underlies not only early alcohol use, but also a number of other behavioral and psychological problems that precede early drinking. That study suggests that the common vulnerability to these kinds of problems is, at least in part, biologically based.

Together, these kinds of studies are providing information that can lead to preventive interventions. Whether or not early drinking leads to alcoholism, alcohol use by children and teens is associated with immediate harm, such as injury, and should be prevented. If further research indicates that early alcohol use does, in fact, lead to alcoholism later in life – an unknown, at this point, – such preventive efforts will have much more far-reaching benefits.

Rose RJ, Dick DM, Viken RJ, Pulkkinen L, Kaprio J: Drinking or abstaining at age 14? A genetic epidemiological study. Alcoholism: Clinical and Experimental Research 25(11): 1594-1604, 2001.

## A Type of Alcohol Blocks Mechanism that Contributes to Fetal Alcohol Syndrome

*Background*: There are several kinds of alcohol, all of whose molecules are variations of the same core structure. Ethanol, the kind of alcohol in beverages, often damages fetuses exposed to it as a result of maternal drinking. The most serious consequence of this damage is fetal alcohol syndrome (FAS), a birth defect that includes permanent behavioral and neurologic deficits, such as mental retardation.

One of the ways that ethanol damages the fetus is by preventing cell adhesion, the stage of development when specialized cells stick to each other to form the various tissues of the body. During this and other stages, temporary cells die at very specific, synchronized times, to make way for mature cells – the normal process of apoptosis. Besides disrupting cell adhesion, ethanol also increases apoptosis inappropriately, which can have devastating consequences.

Researchers theorize that ethanol molecules prevent cell adhesion by occupying the "docking sites" where adhesion molecules called "L1" on cell surfaces normally would bind to each other. Under normal circumstances, when L1 molecules bind to each other, they enable the cells on which they reside to join together. When these cells can't bind to each other because ethanol molecules are interfering, apoptosis results. In other words, when the cells should be joining together to form tissues, ethanol causes them to die, instead. In previous experiments, another kind of alcohol, octanol, kept ethanol from thus thwarting L1 cell adhesion in nerve-cell cultures.

In the study described here, investigators asked if blocking ethanol's disruption of L-1 cell adhesion with octanol translates into fewer physiologic and pathologic deficits in the fetus. The investigators exposed cultured mouse embryos to ethanol at 8 days of development, a stage when alcohol-induced damage is known to occur. The embryos' growth was stunted, and apoptosis increased in cells that eventually form the nerve tissue that develops outside the brain. Then the investigators blocked the effects of ethanol with octanol.

*Advance*: Octanol dramatically decreased ethanol-induced growth reduction and cell death in 8-day-old mouse embryo cultures.

*Implications*: Although exposure to ethanol in the womb is the leading nongenetic cause of mental retardation, clinicians have no treatments to prevent its damage. Treating ethanol-exposed fetuses with octanol to prevent birth defects isn't an option, since octanol itself can be toxic. However, octanol shows a striking ability to salvage L-1 cell adhesion from ethanol's disruption and to prevent excessive apoptosis in cells known to be vulnerable. Isolating the mechanism through which octanol does this holds promise for design of compounds that can safely block ethanol's damaging effects.

Chen S-Y, Wilkemeyer MF, Sulik KK, Charness ME: Octanol antagonism of ethanol teratogenesis. FASEB Journal (express article). Published on-line, May 18, 2001.

## College Students Suffer Major Consequences From Alcohol Misuse

*Background*: Alcohol misuse by college students generally comes to the public's attention only when the occasional media headline announces a student's death from an alcohol overdose. In reality, alcohol misuse underlies a much larger number of student deaths from causes other than overdose and is linked to an even greater number of injuries and assaults, sexual and otherwise. It affects not only students who drink, but also the abstainers around them, as well as the communities in which they reside. Property damage, encounters with the justice system, and derailed academic careers are among other consequences of student alcohol misuse.

About 40 percent of college students at two-year and four-year colleges said they had engaged in episodes of heavy drinking (four or five drinks in a row, depending on the study and the gender of the student) at least once in the two weeks prior to being surveyed. One study also found that 23 percent of four-year students had engaged in this kind of drinking at least three times in the previous two weeks. The study described here focused not on the amount and frequency of drinking by college students, but on estimating the consequences of their drinking.

*Advance*: In 1998, an estimated 1,400 college students died from unintentional injuries, including car crashes, related to alcohol use. More than 2 million drove under the influence of alcohol, and 3 million rode with a driver who had been drinking. More than 500,000 full-time students in four-year schools sustained unintentional injuries that involved alcohol, and more than 600,000 were assaulted by another student who had been drinking.

*Implications*: The estimates in this study underscore the need for rational, science-based strategies to prevent harmful drinking in this vulnerable population. The NIH convened a Task Force of college presidents and researchers, who assessed what was known about college drinking and commissioned studies that would lead to practical recommendations for college administrators. The primary recommendation that emerged calls for comprehensive interventions simultaneously directed at individual students, entire campuses, and college communities. Panel reports and review papers commissioned by the Task Force will provide additional guidance for college presidents, program planners, and researchers.

Hingson RW, Heeren T, Zakocs RC, Kopstein A, Wechsler H: Magnitude of alcohol-related mortality and morbidity among U.S. college students ages 18-24. Journal of Studies on Alcohol 63: 136-144, 2002.

## Receptor Appears to Protect Against Risk of Alcohol Abuse

*Background*: Alcoholism is more than a matter of will and environmental influences; biological factors play a key role in the development of the disease. One of these biological factors is the neurotransmitter dopamine, a chemical that transmits signals to nerve cells in the brain. It is among the substances whose activities in the nervous system ultimately influence future desire to drink. Dopamine-transmitting cells that project into an area of the brain called the "nucleus accumbens" have been implicated in this process.

Alcoholism changes the concentration of receptors, on nerve cells, to which dopamine binds to exert its actions. Brains of alcoholics contain lower-than-normal levels of the dopamine receptor DRD2. In this study, scientists asked how increasing the concentration of DRD2 receptors in the nucleus accumbens would affect rats' (1) preference for alcohol, compared to water, and (2) alcohol intake. Investigators introduced the gene for DRD2 into rats' nucleus accumbens, resulting in overproduction of the receptor.

*Advance*: Induced overproduction of the DRD2 dopamine receptor, in the nucleus accumbens, dramatically reduced alcohol consumption in alcohol-preferring rats previously trained to self-administer alcohol. When DRD2 production returned to normal levels, so did the rats' level of alcohol consumption.

*Implications*: For the first time, scientists have shown that induced overproduction of the DRD2 receptor reduces alcohol intake. This finding suggests that the receptor protects against development of alcohol abuse, and lends strength to the concept that the DRD2 receptor could be a target for design of alcoholism treatments.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Hiroyuki I, Roth G, Ingram DK, Hitzemann R: Overexpression of dopamine D2 receptors reduces alcohol self-administration. Journal of Neurochemistry 78: 1094-1103, 2001.

## Shared Pathology Appears to Precede Early Drinking, Alcoholism, and Other Behavioral Disorders

*Background*: NIH researchers recently discovered a striking association between early age at first alcohol use and development of alcoholism at some point in life. This finding raised another question: Is early alcohol use *per se* a cause of alcoholism, or are both alcoholism and early initiation of drinking reflections of some other childhood vulnerability that underlies a variety of problems?

To explore this question, investigators studied the association between a number of signs of psychopathology and behavioral disorders and age at first drink. They also studied the association between early onset of drinking and a brainwave called "P3," which, when it appears in lower amplitudes than normal, is a known risk marker for alcoholism.

*Advance*: Early age at first drink –11 to 14 years of age – correlated with signs of psychopathology and behavioral disorders, such as attention-deficit disorder and impulsiveness, that appeared in early childhood, before the first drinking experience. In addition, adolescents who began drinking early were more likely than others to have reduced P3 brainwave amplitude.

*Implications*: A particularly suggestive aspect of these findings is that the signs of psychopathology and impulsive behaviors researchers measured – signs like nicotine and drug dependence, antisocial personality disorder, and behavioral conduct disorder – *predicted* which 11-year-olds would try alcohol by age 14. This indicates that these behaviors pre-dated the early drinkers' alcohol use, strengthening the case for a common vulnerability that underlies a range of problems, including both early drinking and alcoholism. The association between reduced P3 amplitude and early onset of drinking suggests that the common vulnerability that appears to underlie these various problems may be, at least in part, physically based.

Even though these findings suggest a common basis for an array of problems, they don't necessarily exclude early drinking itself as a factor that contributes to subsequent development of alcoholism. In addition, young people who drink are at risk of the harm associated with drunk driving, risky sexual behavior, and violence, regardless of why they drink. Other research also suggests that alcohol interferes with neurological development in adolescents. For these and other reasons, preventing children from drinking remains paramount. The challenge these findings raise for researchers is to definitively establish that there is a common basis for the wide range of problems examined in this study and to identify the mechanisms that underlie it. In so doing, they will identify potential targets for pharmaceutical or behavioral interventions.

McGue M, Iacono WG, Legrand LN, Malone S, Elkins I: Origins and consequences of age at first drink. 1. Associations with substance-use disorders, disinhibitory behavior and psychopatholgy, and P3 amplitude. Alcoholism: Clinical and Experimental Research 25(8): 1156-1165, 2001.

**Source of Free Radicals Found and Blocked in Alcoholic Liver Disease**

*Background*: Alcoholic liver disease is the leading cause of cirrhosis deaths in the United States. Scientists are discovering a number of mechanisms through which chronic, heavy alcohol use leads to liver damage. In this study, they focused on reactive oxygen species (ROS), molecules that are beneficial in some cases, but damaging in others. (Most people recognize ROS by the more popular name "free radicals.")

Before this study, scientists knew that alcohol generates ROS in hepatocytes, the main cells of the liver, and that this is one of the ways through which alcohol damages liver tissue. They also knew that CYP2E1, an alcohol-metabolizing enzyme, generates ROS. Another known fact was that fibrosis, a stage of alcoholic liver disease, leads healthy liver tissue to become nonfunctional scar tissue. In fibrosis, specialized liver cells – hepatic stellate cells – become active. These activated cells then produce more than normal of the protein matrix of the liver, especially collagen, setting the stage for scar formation.

In this study, scientists asked how these events are linked; how hepatic stellate cells become activated in the presence of alcohol, leading to fibrosis, and whether ROS generated by the alcohol-metabolizing enzyme CYP2E1 initiate and perpetuate this process. They incubated hepatic stellate cells from rats with (1) human liver cells that produce CYP2E1 and (2) human liver cells that don't produce CYP2E1.

*Advance*: Hepatic stellate cells incubated with CYP2E1-producing human liver cells made more collagen and ROS than human liver cells that don't produce CYP2E1. The stellate cells incubated with CYP2E1-producing hepatocytes also underwent other changes consistent with fibrosis. Treating these altered stellate cells with the following substances reduced their production of excess collagen: antioxidants, compounds that inhibit CYP2E1, or a substance that changes the genetic blueprint for production of CYP2E1.

*Implications*: An alcohol-metabolizing enzyme, CYP2E1, not only disrupts function of the main cells of the liver, but also produces ROS that activate specialized liver cells, leading to fibrotic changes. The fact that antioxidant treatment reduced these pathologic changes has implications for medication design. It is important to note that the mechanism described here isn't the only one that contributes to alcoholic liver disease. Investigators are making significant headway in identifying other mechanisms, which also hold potential as targets for medication design. Each mechanism scientists identify adds to the total picture of how alcohol damages the liver.

Nieto N, Friedman SL, Cederbaum AI: Stimulation and proliferation of primary rat hepatic stellate cells by cytochrome P450 2E1-derived reactive oxygen species. Hepatology 35(1): 62-73, 2002.

**Alcohol Researchers Find a Likely Cause of Cirrhosis's Dangerous Blood-Vessel Dilation**

*Background*: For the first time, scientists have identified a mechanism underlying blood-vessel dilation – "vasodilation" – that occurs in liver cirrhosis. Vasodilation helps maintain normal blood pressure, but in advanced cirrhosis, it is extreme and contributes to cirrhosis's high death rate.

Vasodilation leads, for example, to dangerously low blood pressure and potentially lethal fluid accumulation. It also contributes to a glut of blood in structures that support the liver, engorging its veins and arteries and the veins of the esophagus. This causes localized high pressure in these vessels, creating a risk of rupture and fatal hemorrhage. Paradoxically, the extremely low blood pressure that cirrhotic vasodilation induces in the rest of the body can't support life functions. Standard treatments that raise blood pressure by constricting blood vessels don't work in cirrhosis patients. Scientists have tested other compounds meant to counteract molecular mechanisms hypothesized to cause the vasodilation of cirrhosis, without success. Mechanisms that scientists hadn't targeted in these attempts, until the study described here, appeared to be involved.

In leading up to this study, researchers found that (1) a toxin from bacteria in the intestines enters cirrhosis patients' blood. This kind of toxin in the blood results in low blood pressure. (2) The pressure drop occurs partly through the actions of CB1 receptors, binding sites for naturally occurring molecules called "endocannabinoids." (3) Cells in blood-vessel walls contain $CB_1$ receptors; when endocannabinoids bind to them, low blood pressure due to vasodilation results. Together, these findings suggested a link between $CB_1$ receptors and vasodilation in cirrhosis.

In this study, scientists gave cirrhotic rats a compound (SR141716A) that blocks $CB_1$ receptors. If stopping $CB_1$ activity also stopped the blood-pressure problem, $CB_1$ would be implicated. The investigators went a step further. The toxin in cirrhosis patients' blood causes immune cells (monocytes), also in the blood, to produce endocannabinoids. Researchers asked if monocytes are the source of excess endocannabinoids that reach $CB_1$ receptors in cirrhotic patients.

*Advance*: (1) The $CB_1$ blocker raised blood pressure in cirrhotic rats and reduced blood engorgement of the liver's vessels. (2) Monocytes (the immune cells) contained elevated levels of the endocannabinoid anandamide in cirrhotic humans and rats. (3) When rats that *didn't* have cirrhosis were injected with monocytes from cirrhotic rats or humans, their blood pressure dropped. The $CB_1$ blocker reversed this low blood pressure. (4) In cirrhotic human livers, cells that line blood vessels had three times as many $CB_1$ receptors as did those of noncirrhotic livers.

*Implications:* Each of the angles from which these researchers approached their hypothesis strengthened the case for the CB1 receptor/endocannabinoid system as underlying vasodilation in cirrhosis. This elegant work provides a new paradigm for development of treatments for a serious, costly medical phenomenon, and was published in the prestigious journal *Nature Medicine.*

Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, Wang L, Mirshahi F, Khanolkar AD, Makriyannis A, Urbaschek R, Garcia Jr. N, Sanyal AJ, Kunos G: Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. Nat Med 7(7): 827-832, 2001.

## Moderate Alcohol Use During Year before Heart Attack Linked to Better Outcome

*Background*: Evidence is mounting that moderate alcohol use decreases risk of heart disease, the Nation's leading cause of death. However, we know less about whether or not moderate drinking has a protective effect in people who already have heart disease. A new finding suggests that it does.

Investigators in the from the Determinants of Myocardial Infarction Onset Study examined lifestyle, socioeconomic, and biologic factors in 1,935 men and women soon after they had suffered heart attacks, excluding patients with a history of alcoholism. The researchers included questions about alcohol use in their interviews, and followed patients for periods ranging from 2.2 years to 5.4 years.

*Advance*: Compared to heart-attack patients who abstained from alcohol, those who had up to six alcohol-containing drinks a week in the year before their heart attacks had a lower rate of death from any cause. Patients who had seven or more drinks a week (but remained within the moderate-drinking range) during the year before their heart attacks had an even lower death rate. Results were similar for cardiovascular-related death. These patterns held true regardless of the kind of alcohol the study participants had used.

*Implications*: People who abstain from light or moderate drinking might benefit from additional medical attention after they've had a heart attack. However, this finding does not mean that everyone should drink in an attempt to prevent heart disease or to improve their chances of surviving a heart attack. Some people are at higher risk than others of becoming alcoholic if they begin to drink. Heavy alcohol use can damage any organ in the body, including the brain and liver, and it's linked to some kinds of cancer.

As we discover biological and behavioral markers that tell us who is predisposed to alcohol-induced organ damage and alcoholism, clinicians will be able to weigh the risks and benefits of alcohol use for each patient.

Mukamul KJ, Maclure M, Muller JE, Sherwood JB, Mittleman MA: Prior alcohol consumption and mortality following acute myocardial infarction. JAMA 285(15): 1965-1970, 2001.

## Shape of a Brain Structure Predicts Behavior Problems in Adults Exposed to Alcohol as Fetuses

*Background*: Children whose mothers drank heavily during pregnancy may have permanent damage, including neurologic deficits, behavior problems, and physical deformities. The most serious of these birth defects is fetal alcohol syndrome (FAS), the leading nongenetic cause of mental retardation.

Clinicians cannot rely on physical symptoms of FAS, such as its characteristic facial deformities, to diagnose prenatal alcohol exposure. Not all people exposed to alcohol in the womb have these symptoms, and facial abnormalities become less obvious over time. As a result, clinicians sometimes misdiagnose patients, especially adolescents and adults, who have only neurologic and behavior deficits resulting from alcohol exposure before birth. Traditionally, if these kinds of patients were recognized at all, they were diagnosed with "fetal alcohol effects" (FAE).

Clinicians need measuring methods and protocols that will help them identify the various levels of damage that patients exposed to alcohol *in utero* have sustained. Even more pressing is the need for an understanding of exactly what brain structures are damaged and what kinds of problems result from damage to each structure. Recently, the Institute of Medicine recommended that the diagnoses of FAS and FAE be further refined into five categories.

This study examined males 18 and older who had been diagnosed with FAS or FAE and compared them with "normal" males who didn't have either condition. Investigators conducted magnetic resonance imaging (MRI) studies of the brain and asked the subjects to perform neuropsychological tasks with which people prenatally exposed to alcohol tend to have difficulty. The scientists used a new statistical method to arrive at their conclusions.

*Advance*: Neuroimaging studies showed that FAS and FAE patients had more variability in the shape of a brain structure, the corpus callosum, than did the comparison group. For example, the callosum was thinner or thicker than normal in some FAS/FAE patients. In combination, the imaging studies and neuropsychological tasks revealed that excessive thickness or thinness of the corpus callosum was associated with specific behavioral deficits – executive functions, like memory, and motor function, respectively – in FAS/FAE patients.

*Implications*: Using a new statistical method, investigators were better able to identify distortion of the corpus callosum in FAS/FAE patients. Their results suggest that the shape of the corpus callosum holds promise as a predictor of the type of neuropsychological problems that people who were exposed to alcohol in the womb may have. With accurate diagnosis, people with such problems can be recognized and offered educational and other services appropriate to their needs.

Bookstein FL, Streissguth AP, Sampson PD, Connor PD, Barr HM: Corpus callosum shape and neuro-psychological deficits in adult males with heavy fetal alcohol exposure. Neuroimage 15: 233-251, 2002.

## Even Moderate Drinking During Pregnancy May Be Risk to Offspring

*Background*: The link between alcohol abuse during pregnancy and fetal damage is unquestionable. Heavy alcohol use often results in fetal alcohol syndrome (FAS) and is the leading nongenetic cause of mental retardation. More subtle neurological and behavioral deficits, many of them disruptive to learning, memory, and cognition, also may appear as alcohol-related neurobehavioral deficit (ARND).

While the link between heavy alcohol use and fetal damage is clear, less is known about the effect of moderate drinking on the fetus. Scientists recently addressed this issue in a study of the offspring of rhesus-monkey mothers who voluntarily drank one-to-two drinks a day, the equivalent of moderate drinking in humans, during pregnancy.

The researchers also assessed the effects of mild maternal psychological stress during pregnancy, alone or combined with moderate drinking, on the monkeys' offspring. In all, the researchers studied four groups of pregnant monkeys: those that got (1) alcohol only, (2) mild stressors only, (3) both alcohol and stressors, and (4) neither alcohol nor stressors. When the monkeys' offspring reached adolescence, the researchers trained them to perform a cognitive task that required them to use functions that may be impaired by prenatal alcohol exposure: learning, shifting attention, and memory.

*Advance*: Adolescent monkeys whose mothers had taken one-to-two drinks a day during pregnancy had difficulty acquiring a cognitive task and had behavioral disturbances. The worst behavioral outcomes, which included hyperactivity, irritability, and restricted, repetitive behaviors, appeared in the group of monkeys whose mothers had gotten both alcohol and a mild stressor during pregnancy.

*Implications*: Fetal development is similar in humans and rhesus monkeys. With this study of primates, we now have evidence that even social drinking during pregnancy may have lasting effects on cognition, learning, and memory of offspring, and that prenatal stress may promote or exacerbate some of these effects. There are no clinical interventions that prevent alcohol from damaging the fetus or treat the damage once it has occurred. This rhesus model will provide a valuable research tool for testing potential therapeutic approaches.

Schneider ML, Moore CF, Kraemer GW: Moderate alcohol during pregnancy: Learning and behavior in adolescent rhesus monkeys. Alcoholism: Clinical and Experimental Research 25(9): 1383-1392, 2001.

## Gene Regulates Two Behaviors Associated with Alcoholism

*Background*: Some personality traits, as well as biological factors, predict which of us are more at risk than others of becoming alcoholic. For example, alcoholics in the "Type II" category – those who are socially aggressive, engage in novelty-seeking behaviors, and start drinking before age 25 – generally are impulsive.

In nerve cells, genes are active players in pathways of molecular reactions that translate into these and other behaviors, although exactly which genes are involved remains elusive. Another behavior that has yielded evidence of a genetic link is alcohol preference. This behavior is reflected in the act of alcohol consumption, itself, versus abstinence from drinking, and in the act of consuming large versus small amounts of alcohol, or avoiding alcohol altogether.

An important regulator of these behavior-influencing molecular pathways in nerve cells is a molecule called "protein kinase C gamma" (PKC ). Recent studies suggest that variations in PKC result in variations in responses to alcohol. In the study described here, scientists asked if the gene that produces PKC affects behaviors associated not only with impulsivity, but also with alcohol preference.

Mice genetically engineered to lack PKC were given a choice of water or alcohol, with unlimited food. To test impulsivity, scientists trained the mice to poke a lever to obtain food, and observed their ability to wait until the right time to poke the lever. Investigators took steps to ensure that the resulting behaviors weren't due to need for calories or inability to learn.

*Advance*: Mice lacking PKC chose to drink significantly more alcohol and were significantly more impulsive than were mice with intact PKC.

*Implications*: The gene that produces PKC appears to be among those that influence alcohol preference and impulsivity, traits associated with Type II alcoholism. As scientists unravel the genetic and molecular pathways that influence drinking behaviors, they identify potential targets for medication design. Pending further research, PKC is a potential candidate.

Bowers BJ, Wehner J: Ethanol consumption and behavioral impulsivity are increased in protein kinase C null mutant mice. J Neurosci 21: 1-5, 2001.

**Brains of Alcoholics Reorganize to Perform Cognitive Tasks**

*Background*: Alcoholics have impairments in memory and attention that are associated with damage to specific regions of the brain. These deficits may persist long after alcoholics quit drinking, despite substantial recovery of brain structure and function. Alcoholics aren't necessarily *un*able to perform tasks requiring memory and attention skills; they are less able.

In this study, scientists asked what parts of the brain govern performance of memory and attention tasks in alcoholic men, compared with nonalcoholic men. Normally, an area of the brain called the prefrontal cortex controls cognitive functions like problem solving and memory of spatial relationships. The investigators used functional magnetic resonance imaging (fMRI) to find out what areas of the brain became active while study participants performed attention and visual memory tasks.

*Advance*: Alcoholics and nonalcoholics both were able to perform the attention and memory tasks. However, fMRI showed that alcoholics were less able to engage the frontal-lobe systems of the brain that normally govern performance of these kinds of activities. Meanwhile, unimpaired brain regions that weren't active in nonalcoholics were active in alcoholics.

*Implications*: When systems that normally govern attention and memory are damaged by chronic alcohol abuse, the brain appears to draw on some of its other, less impaired systems to perform these functions. The possibility that alcoholics' brains reroute their circuits, to compensate for damaged brain regions, suggests a new avenue for corrective training – therapy that would promote use of brain regions unimpaired by alcohol to perform essential cognitive functions.

Pfefferbaum A, Desmond JE, Galloway C, Menon V, Glover GH, Sullivan E: Reorganization of frontal systems used by alcoholics for spatial working memory: A fMRI Study. NeuroImage 14: 7-20, 2001.

## Increased Use of Cigarettes, Alcohol, and Marijuana Among Manhattan Residents Following September 11th Attacks

*Background:* Exposure to stress is among the most common of human experiences, and it can also be one of the most powerful triggers of substance abuse in vulnerable individuals, and of relapse in former addicts. The attacks on September 11th, 2001 were among the most horrific events experienced in the recent past, and many people continue to struggle with the emotional impact of that event, and with the uncertainty of what lies ahead. Assessing the impact of September 11th and its aftermath on alcohol and drug use and other mental health issues is important in order to appropriately allocate needed resources for treatment and prevention of further disability and distress. Moreover, the lessons learned following this and other disasters are valuable in helping the country prepare for future human-caused and natural disasters, and, more generally, for increasing our understanding of the role that trauma plays in substance use and abuse.

*Advance:* Using a random-digit dial telephone survey, researchers examined the prevalence of increased cigarette smoking, alcohol consumption, and marijuana use in individuals living in proximity to the World Trade Centers site. The study was conducted 5-8 weeks after the attack and included a sample of almost 1000 adults. Comparisons were made between the week before September 11th and the week before the survey was conducted. An increase in substance use was found in 29 percent of respondents. Almost 10 percent reported an increase in smoking (in some cases by a pack a day); 25 percent increased their alcohol intake; and 3 percent increased their use of marijuana. The rates of increased substance use were much higher in individuals who also reported symptoms of post-traumatic-stress disorder and/or depression, as well as those who experienced panic attacks around the time of the event.

*Implications:* Increased rates of substance use were reported by those in proximity to the World Trade Center in the weeks following September 11th compared to the week before the attacks. While this time point may be too soon to evaluate whether dependence and addiction rates also increase, the study highlights the importance of including substance use prevention and treatment in disaster planning and responding. Public education efforts should target this issue and health care practitioners need to be vigilant in assessing and responding to the substance abuse and mental health impact of a disaster. The high rates of co-occurring mental disorders and substance use suggest that more research should focus on uncovering the mechanisms of and relationships between traumatic events, stress, psychopathology, and substance use and dependence.

Vlahov, D., Galea, S., Resnick, H., Ahern, J., Boscarino J.A., Bucuvalas, M., Gold, J. Kilpatrick, D. Increased use of cigarettes, alcohol, and marijuana among Manhattan, New York, residents after the September 11th terrorist attacks. American Journal of Epidemiology 155(11): 988-996, 2002.

## Binge Use of Ecstasy is Toxic to the Heart

*Background*: Abuse of 3,4,-methylenedioxymethamphetamine (MDMA; Ecstasy) has been increasing at alarming rates. This illicit drug has become particularly popular at raves, parties, and dance clubs, in part because its stimulant properties enable users to dance for extended periods of time. MDMA can cause a number of problems including heart attacks, seizures, and even death. Research has also has shown that MDMA can be a significant neurotoxin. The typical patterns of MDMA abuse range from a single dose to heavy binges over a period of a few days followed by a period of abstinence. These patterns of use are often repeated. Although MDMA has the potential to produce cardiovascular toxicity, little is known about its specific effects on cardiovascular functioning, especially following repeated or binge use.

*Advance*: Researchers used rats to study the impact of binge administration of MDMA on heart rate, arterial pressure, and the heart muscle. Rats were given MDMA twice a day for four days, followed by a ten day drug free period. This regimen was repeated three times to simulate drug-taking patterns seen with humans. This binge pattern caused significant alterations in cardiovascular function that increased with each subsequent binge. For example, the first binge caused an increase in heart rate followed by a slowing of the heart and a reduction in blood pressure. By the third binge, the reduction in heart rate and blood pressure were considerably greater than seen in the first binge, indicating that there is a cumulative effect of MDMA on heart function. In addition, over the course of several MDMA binges, the researchers observed an increased potential for MDMA to generate cardiac arrhythmias. Finally, the binge administration was found to damage the heart muscle itself. The hearts of rats exposed to only one MDMA binge were not significantly different from normal controls. However, the hearts of rats exposed to three MDMA binges showed damage to the heart tissue that included necrosis, or destruction of heart muscle cells.

*Implications*: This study in rats clearly shows that repeated binge use of MDMA can have serious implications for heart function that increase with each subsequent binge. This cardiac toxicity can include damage to the heart muscle itself and may account for the cardiovascular failure and heart attacks that are seen with some MDMA users. These findings also indicate that emergency room personnel and other treatment providers should be aware of the increased potential for cardiac problems among patients who are binge MDMA users.

Badon LA, Hicks A, Lord K, Ogden BA, Meleg-Smith S, Varner KJ: Changes in Cardiovascular Responsiveness and Cardiotoxicity Elicited During Binge Administration of Ecstasy. JPET 302(3): 898-907, 2002.

**Although Brain Structure May Have capacity to Recover from Methamphetamine with Protracted Abstinence; Brain Function May Not**

*Background:* The stimulant methamphetamine continues to be abused throughout the world. Not only is methamphetamine highly addictive, but it has been shown in both animals and in humans to damage brain cells that contain the neurotransmitter dopamine, as indicated by decreases in the number of dopamine transporters (DAT) in the brain. Neural changes can persist several weeks, months, and years following the last drug exposure. It is unclear whether the DAT losses in methamphetamine abusers will place these individuals at increased risk for neurodegenerative diseases such as Parkinson's Disease as they age.

*Advance:* Using positron emission tomography (PET), researchers measured levels of dopamine transporters in the brain of methamphetamine abusers and controls. Five long-term methamphetamine abusers were tested twice during the study. Once, approximately one month after abstinence from drugs, and again at about nine months of abstinence.

Five additional methamphetamine abusers, who were abstinent for nine months, were also tested. The researchers found that DAT availability was significantly increased from short to protracted abstinence in certain regions of the brain of those tested twice, suggesting that DAT recovery is in part a function of the length of time the methamphetamine abusers can stay off the drug. This is the first evidence in humans that the dopamine transporter losses from chronic methamphetamine exposure can recover significantly with long-term abstinence. However neuropsychological function did not recover with protracted abstinence, suggesting that although there is recovery of DAT levels there is not a parallel improvement in function.

*Implications:* Further studies in larger samples are needed to assess whether recovery of dopamine transporters with protracted abstinence is associated with recovery of neuropsychological function. The findings, however offer cautious optimism, for the treatment of methamphetamine abusers suggesting that protracted abstinence and proper rehabilitation may reverse some of the methamphetamine-induced alterations in brain dopamine terminals. This finding also has tremendous implications not only for drug addiction researchers, but those studying other neurodegenerative disorders such as Parkinson's and Alzheimer's.

Volkow ND, Chang L, Wang GJ, Fowler JS, Francheschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding Y, Logan J: Loss of the Dopamine Transporters in Methamphetamine Abusers Recovers with Protracted Abstinence. J Neurosci 21(23): 9414-9418, 2001.

## Prenatal Exposure to Cocaine Can Result in Significant Deficits in Mental Skills of Toddlers

*Background:* Maternal use of cocaine during pregnancy remains a significant public health problem, particularly in urban areas and among women of low socioeconomic status. It is estimated that approximately 1 million children have been born prenatally exposed to cocaine since the mid 1980s. There have been very few longitudinal studies that have followed the development of children prenatally exposed to cocaine.

*Advance:* This study followed a cohort of 218 cocaine-exposed infants recruited from a sample prospectively screened at birth at a large urban county hospital and 197 non-exposed infants from the same population. The cocaine-exposed infants were compared with the non-exposed infants in regard to cognitive and motor developments until two years of age. Each child was tested at 6.5 months, 12 months and 24 months for developmental progress. A widely used standardized assessment tool of infant development yielded information about mental development and motor coordination. After controlling for confounding variables, such as minority race, socioeconomic status, and poor prenatal care, the researchers found that cocaine-exposed children performed more poorly on the mental assessment than those infants non-exposed. There was a 6-point deficit in the scores between the 2 groups, with cocaine-exposed children twice as likely to have significant delays in mental development. Almost 14 percent of the cocaine-exposed infants had scores in the mental retardation range, almost 5 times higher than expected in the general population. There did not appear to be any major differences, however, in the motor skills development that can be attributed to cocaine.

*Implications:* The cocaine-exposed children in this study were shown to have significant cognitive deficits and a doubling of the rate of developmental delay during the first 2 years of life. Because 2-year outcomes are predictive of later cognitive outcomes, it is possible that these children will continue to have learning difficulties at school age. It is critical that we do not stigmatize, but rather provide assistance to these children to enhance their cognitive development.

Singer LT, Arendt R, Minnes S, Farkas K, Salvator A, Kirchner HL, Kliegman: Cognitive and Motor Outcomes of Cocaine-Exposed Infants. JAMA, 287(15): 1952-1960, 2002.

## Social Factors Can Change Neurobiology and Affect Vulnerability to Drug-Taking

*Background:* While it is well-established that social factors can influence susceptibility to drug abuse, the brain mechanisms responsible for this effect are unknown. Data from a variety of animal and human studies have directly implicated the neurotransmitter dopamine (DA) in the rewarding properties of both natural reinforcers (e.g., food, sex) and drugs of abuse. Moreover, imaging studies have shown marked differences in DA systems in the brains of human drug abusers compared to controls that do not use drugs. However, it is unclear whether some or all of these DA effects were present prior to drug use and could relate to vulnerability to addiction, or whether they are the consequences of substance abuse, or both. The development of an animal model to investigate these questions could prove enlightening regarding mechanisms of environmental impact on drug abuse and potential targets for intervention and prevention strategies.

*Advance:* A study was conducted in non-human primates using positron emission tomography (PET) imaging to assess dopamine receptor function in relation to individual vs. social housing conditions. Male monkeys were initially housed alone for 1.5 years before being placed in a group environment with 3 other males. A social dominance hierarchy tends to form under the latter conditions, and each monkey's behaviors (e.g., aggression, grooming, social contact) were characterized to determine the monkey's position in the hierarchy. DA D2 receptors were imaged before and at least 3 months after the group housing occurred. Although no differences in D2 receptors were observed within the groups before social housing, those monkeys that became dominant showed increases in their D2 receptor binding compared to their own previous levels, and to the subordinate monkeys. When subsequently given access to cocaine, the *subordinate* monkeys showed greater overall intake of cocaine, and also self-administered low doses, which did not support drug-taking in the dominant animals.

*Implications:* In human drug abusers, decreased D2 receptor availability has been reported for a variety of substances of abuse. In one study of non-drug-abusing humans, low levels of D2 receptors were shown to be predictive of a pleasurable response to methylphenidate (Ritalin). Together with the results in non-human primates, these data suggest that D2 receptor levels (particularly in certain brain regions) may represent a vulnerability marker for future substance abuse, and one that is amenable to change either through drug exposure or changes in drug environment. A remarkable degree of plasticity was found for D2 receptors in adult monkeys following just 3 months of exposure to a new social context. It is possible that the changes observed in these monkeys were related to their return to an environment in which they could control such factors as social contact, mobility within the environment, and access to food and other resources. These data provide a basis for testing the utility of social, behavioral, or even pharmacological interventions that could achieve similar results in humans in order to prevent drug abuse and relapse to addiction.

Morgan, D., Grant, K.A., Gage, D., Mach, R.H., Kaplan, J.R., Prioleau, O., Nader, S.H., Buchheimer, N., Ehrenkaufer, R.L., & Nader, M. A. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 5(2):169-174, 2002.

## Mutant Gene Associated with Drug Abuse

*Background:* Drug abuse and addiction are complex neurobehavioral disorders that have both environmental and genetic influences. It is estimated that genetic factors account for 40-60 percent of the risk of abuse and addiction, yet the precise identities of human genes that increase vulnerability are unknown. Advances in our understanding of the neurobiological mechanisms underlying abuse and addiction have given researchers many promising candidate genes that may play a role in increasing an individual's vulnerability. New findings indicate that a mutation in an enzyme known as fatty acid amide hydrolase (FAAH), may play a role in regulating the brain's cannabinoid system. Because the cannabinoid system has been shown to influence sensitivity to other drugs of abuse, the gene for FAAH is a promising candidate for conferring vulnerability to drug abuse and addiction.

*Advance:* Researchers sequenced the FAAH gene from the DNA of problem drug users and non-drug users. They were able to identify variation in the sequences, or single nucleotide polymorphisms (SNPs). In characterizing whether these changes in DNA sequence altered the protein structure, the researchers found that a variation at one site (at amino acid number 385, a cytosine was converted to adenosine) was significant in that it created a potentially functional mutation in the protein structure. In comparing the substance-abusing patients with the non-drug users, researchers found that the presence of the 385A mutation is strongly associated with self-reported street drug use and problem drug and alcohol use.

*Implications:* Addiction is a complex disorder that is influenced by many variables, including genes. The identification of the FAAH mutation that is strongly associated with drug use offers a potential diagnostic predictor of an individuals risk for drug-related disorders.

Sipe JC, K Chiang K, Gerber AL, Beutler E, Cravatt BF: A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Nat Acad Sci 99(12): 8394-8399, 2002.

## Prenatal Exposure to Methamphetamine Enhances the Toxic Effects of Methamphetamine in Adult Male Rats

*Background*: Methamphetamine is a highly addictive stimulant drug that is abused in epidemic proportions in many parts of the world. Use by women of child-bearing age has become a major public health concern because of the possible risks to the exposed fetus. Studies in animals have shown that methamphetamine can produce profound and long-lasting neurotoxic damage to the brain, particularly to neurons that contain the neurotransmitter dopamine. It is not known, however, what impact methamphetamine has on the dopamine system of the developing brain or whether there are any long-term health consequences associated with prenatal methamphetamine exposure.

*Advance*: Researchers examined whether adult animals that had been exposed to methamphetamine prenatally might be more susceptible to the neurotoxic effects of the drug. Pregnant rats were given methamphetamine at various stages of pregnancy in doses that were determined to be neurotoxic to adult animals. The resulting rat pups were weaned normally and allowed to develop into adults when they were exposed to a range of methamphetamine doses Their brains were examined for changes in the dopamine system. Results showed that male rats had greater reductions in dopamine markers in several brain areas compared to animals that had not been exposed prenatally to methamphetamine. This effect was not as marked in most brain regions of female rats. Reductions in these dopamine markers are known to be associated with damage to nerve cells that contain the neurotransmitter dopamine. The greater reduction of these markers in male rats that had been exposed *in utero* to methamphetamine indicates that methamphetamine may cause changes in the developing brain that result in an increased sensitivity to the toxic effects of the drug.

*Implications*: Although many women of child-bearing age are abusing methamphetamine, little is known about the short or long-term impact of its use on the developing fetus. This study showed that prenatal exposure to methamphetamine increased the sensitivity of adult male rats to the neurotoxic effects of methamphetamine. These findings raise serious concerns that male methamphetamine abusers may have an enhanced neurotoxic risk to this drug, and perhaps other drugs of abuse also, if they were exposed to methamphetamine *in utero*. Damage to the dopamine system may predispose individuals to a variety of health problems, including Parkinson's disease and other neuropsychiatric disorders which result from abnormalities of dopamine function. Further studies are needed to determine the precise mechanisms for the observed effects and to develop treatments aimed at reversing brain changes that happen *in utero* following maternal methamphetamine abuse.

Heller A, Bubula N, Lew R, Heller B, Won L: Gender-Dependent Enhanced Adult Neurotoxic Response to Methamphetamine Following Fetal Exposure to the Drug. The Journal of Pharmacology and Experimental Therapeutics 298(2):769-779, 2001.

## Understanding Addiction at the Molecular Level

*Background:* Relapse to drug abuse is often triggered by reminders or cues associated with previous drug taking. Thus, researchers have been exploring the links between memory and addiction on a number of levels. This includes understanding how changes in gene expression affect learning and memory. Several gene sequences have been identified that serve as binding sites for proteins, commonly called "transcription factors" which can change gene expression. Understanding how these transcription factors may work together to change neuronal gene expression can offer insight into drug addiction as well as learning and memory processes, and may ultimately lead to the development of new treatment medications for addiction.

*Advance:* Using the larva of the fruit fly Drosophilia, researchers have provided new insight into how molecules may control addiction, memory formation, and brain plasticity. By creating strains of Drosophilia lacking important transcription factors, it was determined that the transcription factor, AP1 positively regulates both the number of synapses formed between neurons and increases the strength of those synapses. Additionally, it was also found that AP1 could influence the levels of another common transcription factor, CREB, which has previously been implicated in synaptic plasticity. These findings suggest that AP1 is more important in regulating the formation of new synapses than previously thought.

*Implications:* By understanding the factors promoting neuronal plasticity, researchers gain new insights not only into brain development but also into the processes underlying memory formation. Memory processes are thought to be an important component in the development of addiction and contribute to the relapse that often occurs among individuals who are attempting to quit drug use.

Sanyal S, Sandstrom DJ, Hoeffer CA, and Ramaswami M. AP1 Functions Upstream of CREB to Control Synaptic Plasticity in Drosophila. Nature 416: 870-874, 2002.

## 3-D Visualization of Gene Expression in the Brain

*Background:* Research has demonstrated that neuropsychiatric disorders, while having many unique features, also have many commonalities. For example, perturbations in the brain's dopamine system appear to underlie disorders such as Parkinson's Disease (PD), schizophrenia, and addiction. Genetics also appear to importantly contribute to determining who will develop these disorders, yet because of their complexity, it has been difficult to identify the relevant genes. Advances in new technologies such as microarrays and gene chips are allowing researchers to systematically analyze large numbers of genes but they give little information on where in the brain these changes are occurring. The development of a technology that could both assess changes in gene expression and pin-point those changes in the brain would greatly facilitate our understanding of a number of neuropsychiatric diseases including addiction.

*Advance:* Researchers have developed a new method called "voxelation" to investigate changes in gene expression in a mouse model of PD. A "voxel" is a 3-dimensional image element that when put together with adjacent voxels can create a spatial map of the brain. To induce PD, mice were administered high doses of methamphetamine which resulted in 45 percent losses in dopaminergic neurons in the substantia nigra of the brain. Brains from PD and control mice were divided into 40 voxels and gene expression was assessed using a microarray in each of the voxels. This enabled the researchers to create spatial maps of gene expression of the PD and control mice. The analysis revealed a common network of co-regulated genes as well as a number of genes differentially expressed in several brain regions in the PD mice.

*Implications:* The identification of regionally co-regulated genes will give investigators new information on the factors regulating brain development. Additionally, identifying the genes that are impacted by diseases such as PD, depression, addiction and others, will offer important starting points for the development of novel therapies.

Brown VM, Ossadtchi A, Khan AH, Yee S, Lacan G, Melega WP, Cherry SR, Leahy RM and Smith DJ: Multiplex Three-Dimensional Brain Gene Expression Mapping in a Mouse Model of Parkinson's Disease. Genome Research, 12: 868-884, 2002.

## Anthrax Toxin Structure Solved

*Background:* Three toxic proteins are critical for the deadly effect of the now-familiar anthrax bacterium, *Bacillus anthracis*. One of these toxins, called protective antigen, allows the other anthrax toxins to enter cells. The second, lethal factor, destroys immune system cells that normally defend the body. This process releases inflammatory molecules that can cause septic shock and death. The third toxin, called edema factor, causes potentially lethal swelling and fluid buildup in the body. By itself, edema factor can be deadly. It also makes lethal factor 10 to 100 times more potent.

*Advance:* Scientists already knew the shape and features of the anthrax protective antigen and lethal factor toxins. This year, by solving the structure of edema factor, they completed the detailed, three-dimensional picture of the deadly triumvirate. Edema factor is harmless until it binds to a molecule called calmodulin. The new study reveals that edema factor changes its shape dramatically when it binds to calmodulin, creating a pocket in which it carries out the chemical reactions responsible for its toxic effects.

*Implications:* Because it contains a deep, narrow pocket, the activated edema factor appears to be an ideal drug target. By designing a small molecule to clog this pocket, pharmaceutical scientists may be able to develop a drug to combat anthrax. Other bacterial diseases rely on proteins similar to edema factor, including whooping cough and a hospital-acquired infection caused by *Pseudomonas aeruginosa*. The structure of edema factor will provide a good starting point for designing new drugs to treat these other diseases as well.

This advance grew out of basic research on cell communication that initially appeared to have little immediate significance for human health. It is now directly confronting a critical public health threat. In the words of the biochemist who led the research: "Three years ago, when we started this project, *Bacillus anthracis* was an obscure agricultural pathogen with interesting biological properties. Now, anthrax is front and center in every clinician's mind. ...We hope this work will quickly lead to new therapies."

Drum CL, Yan S-Z, Bard J, Shen Y-Q, Lu D Soelaiman S, Grabarek Z, Bohm A, Tang W-J: Structural Basis for the Activation of Anthax Adenylyl Cyclase Exotoxin by Calmodulin. Nature 415: 396-402, 2002.

## Stop Cell Death, Help Treat Sepsis?

*Background*: The leading cause of death in critically ill patients is a body-wide infection called sepsis. Nationwide, it strikes 750,000 people every year and kills over 210,000.[1] Sepsis occurs when bacteria leak into the bloodstream, causing widespread damage all over the body. Blood pressure plunges dangerously low, the heart has difficulty pumping enough blood, and body temperature climbs or falls rapidly, in many cases causing multiple organs to fail. In recent years, researchers have come to realize that the gut, or intestinal tract, plays an important role in sepsis. Scientists have found that after a severe infection or injury, cells in the intestinal lining die off in a process called apoptosis.

*Advance*: Researchers now suspect that blocking apoptosis in the intestines of critically ill patients may help prevent death from sepsis. The strategy looks promising in mice, suggesting that it may someday be effective in people. A team of surgeons and basic researchers genetically engineered laboratory mice to produce, in their intestines, large amounts of a cell death-blocking protein called bcl-2. The scientists exposed the experimental mice to the bacterium *Pseudomonas aeruginosa*, which can cause sepsis in susceptible people. Remarkably, 40 percent of the mice with bcl-2 escaped infection and survived, compared to only 4 percent of mice without bcl-2.

*Implications*: The study suggests that minimizing intestinal cell death may prevent death in people with sepsis. Effective prevention and treatments are urgently needed since the death rate from sepsis has climbed more than 90 percent over the last 20 years,[2] costing the nation $16.7 billion per year.[1]

Coopersmith CM, Stromberg PE, Dunne WM, Davis CG, Amiot II DM, Buchman TG, Karl IE, Hotchkiss RS: Inhibition of Intestinal Epithelial Apoptosis and Survival in a Murine Model of Pneumonia-Induced Sepsis. JAMA 287(13): 1716-21, 2002.

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[1] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of Severe Sepsis in the United States: Analysis of Incidence, Outcome, and Associated Costs of Care. Crit. Care Med. 29: 1303-10, 2001.

[2] Murphy SL. Deaths: Final Data for 1998. Natl. Vital Stat. Rep. 48: 1-105, 2000.

## Studies of Iron-Pumping Bacteria May Lead to New Antibiotics

*Background:* The war against drug-resistant bacteria continues to intensify. A few years ago, hospital workers detected strains of *Staphylococcus aureus* – the primary cause of hospital-acquired infections – that are resistant to every known antibiotic medicine. Major killers worldwide such as pneumonia, malaria, tuberculosis, cholera, and gonorrhea are progressively defying all treatment options. And with the ease of international travel, a drug-resistant microbe originating oversees can arrive on U.S. shores within 24 hours. To stem the rising tide of drug-resistant bacteria, scientists are scrambling to design new drugs.

To become and remain infectious, many disease-causing bacteria literally "pump iron." That is, they pump the metal from their hosts' bodies into their cells using proteins in their outer membranes that open and close. These transporter proteins are found in dangerous bacteria such as those that cause cholera, dysentery, blood poisoning, meningitis, and plague.

*Advance:* This year, structural biologists revealed the atomic details of one iron-pumping transporter protein called FecA. According to the new three-dimensional image, FecA looks like a barrel that is open on both ends and plugged in the middle. After iron (and a special carrier protein) enters from the top, this entrance closes behind it, the FecA plug opens, and the iron passes through into the bacterial cell.

*Implications:* A better understanding of how disease-causing bacteria become infectious may lead to new drugs to treat such diseases. Scientists may be able to design novel antibiotics that physically mimic the natural iron carriers. The idea is, the transporter proteins would latch onto the drugs, "think" they contain iron, and actively pump them into bacteria. Once inside, instead of arming the bacteria for infection, the drugs would kill the bacteria. Now that scientists know how the transporter proteins operate, they can try various ways to manipulate them to admit drugs or to keep out iron. More generally, the study improves our understanding of how bacteria obtain essential nutrients.

Ferguson AD, Chakraborty R, Smith BS, Esser L, van der Helm D, Deisenhofer J: Structural Basis of Gating by the Outer Membrane Transporter FecA. Science 295: 1715-1719, 2002.

## Bacteria Study Sheds Light on Cell Communication

*Background*: Scientists have known for years that bacterial cells use molecules on their surfaces called receptors to help them sense and respond to their environment. Somehow, bacteria are able to sense vanishingly small amounts of an environmental signal – like a nutrient – then amplify the signal significantly to prompt a quick reaction, such as moving toward food or away from danger. Until recently, scientists were mystified as to how the signal amplification process takes place.

*Advance*: A team of chemists has uncovered a system bacterial cells use to sense, analyze, and deliver signals to the cell interior. The scientists chemically manufactured multi-pronged molecules that would attach not to one, but to an entire group of cell surface receptors called "chemoreceptors." These synthetic molecules allowed the researchers to control and study cell responses such as bacterial cell motion. The researchers found that bacterial chemoreceptors that sense food and other chemicals in a cell's environment team up in groups to amplify a signal and orchestrate an appropriate response. Chemoreceptors do this, the scientists discovered, by snuggling together on the cell surface into a lattice-type structure that acts sort of like a molecular "nose."

*Implications*: The work may allow researchers to create chemically treated surfaces that repel dangerous microbes on contact. Better knowledge of cell communication among bacteria could also help scientists learn how to dismantle complex "neighborhoods" of communicating bacteria called biofilms that can coat the surfaces of catheters and other medical devices. Biofilms play a role in a variety of diseases, including cystic fibrosis and Legionnaire's disease, and infections caused by these bacterial complexes are notoriously resistant to antibiotics. Researchers suspect that human immune cell receptors work in teams, similar to the behavior of *E. coli* bacteria demonstrated in this study. Further investigations might be able to confirm these guesses and advance progress in understanding the human immune system.

Gestwicki JE, Kiessling LL: Inter-Receptor Communication Through Arrays of Bacterial Chemoreceptors. Nature 415: 81-84, 2002.

## A Protein to Tie up Loose Ends

*Background*: Our genetic material is under constant assault, bombarded by a variety of sources that break the long strands of DNA in our chromosomes. The culprits? Cosmic radiation and energetic molecules, called free radicals, found in sources ranging from smog to nitrite food preservatives. Breaks in DNA can cripple cells' ability to make the proteins they need to survive and – if passed on to subsequent generations of cells – can lead to a host of diseases, including cancer. Cells have repair mechanisms that rejoin broken DNA strands, but sometimes the genetic coding for these repair mechanisms is defective. When this happens, cells cannot manufacture the repair proteins they need. A very serious immune system disorder, severe combined immunodeficiency disease, affects people who inherit a particular type of genetic defect in their cellular repair mechanisms. This disease was featured in the 1976 TV movie, *The Boy in the Plastic Bubble*. Medical researchers located the gene responsible for about 15 percent of severe combined immunodeficiency cases and named it Artemis, after the Greek goddess for the protection of children. But until now, the protein product of the Artemis gene was unknown.

*Advance*: A team of pathology and biochemistry researchers identified the Artemis protein and described how it works. They discovered that it is an enzyme essential for repairing breaks in DNA by trimming away the frayed tails left at randomly broken DNA ends. Other proteins then rejoin the DNA segments. This housekeeping activity is crucial for survival in all cells, and those lacking the Artemis protein quickly accumulate fragmented chromosomes, as more and more of the DNA strands within the chromosomes break. The same group of researchers also discovered that Artemis has an additional function within specialized immune system cells called lymphocytes. These cells use Artemis to shuffle the DNA that makes up immunity genes in order to generate the wide range of antibodies and other immune molecules needed to combat many different bacteria, viruses, fungi and other threats. Without the Artemis protein, these immune system molecules fail to develop, leaving the body vulnerable to any number of diseases and infections.

*Implications*: A drug that temporarily inhibits the activity of Artemis could boost the effectiveness of radiation therapy for cancer patients. The researchers envision that disabling the Artemis protein during radiation treatment would block the ability of cancer cells to repair themselves after being damaged by the radiation. With their increased knowledge of the Artemis protein, the researchers plan to screen for drugs that inhibit the protein. In addition, the new knowledge about Artemis adds to our general understanding of DNA repair mechanisms, a process vital to the survival of our cells.

Ma Y, Pannicke U, Schwarz K, Lieber M: Hairpin Opening and Overhang Processing by an Artemis/DNA-Dependent Protein Kinase Complex in Nonhomologous End Joining and V(D)J Recombination. Cell 108: 781-794, 2002.

## A New Way to Relax – At the Molecular Level

*Background:* Most cells contain motor proteins that control critical activities ranging from cell division to movement. Because motor proteins are necessary for essential cellular functions, defects in the proteins usually cause death during early embryonic development. As a result, cell biologists do not have model systems to study what happens to an organism when motor proteins malfunction. To get around this problem, the scientists harness small molecules to enter cells in laboratory dishes and block a specific motor protein's activity.

*Advance:* A group of researchers used a series of chemical screens to fish out from a library of 16,300 compounds a small-molecule inhibitor of a motor protein called fast skeletal muscle myosin II. This type of myosin controls the muscle contractions that allow humans – and other animals – to run, jump, blink, breathe, and do any other voluntary movement. Other types of myosin control the involuntary muscles in our hearts, blood vessels, and other internal organs. The new inhibitor, nicknamed BTS, blocks fast skeletal myosin with exquisite specificity and potency.

*Implications:* Compounds that inhibit skeletal muscle myosin are excellent for preventing unwanted muscle contractions in biopsied tissues, which interfere with diagnosis. As such, BTS holds great promise for advancing the understanding, diagnosis, and treatment of various muscle disorders. Although any clinical applications of BTS are a long way off, general muscle relaxants are useful during major surgery when patients are under general anesthesia and on respirators. Applying BTS to specific muscles, say in the case of sports injuries, is trickier. The key will be to prevent it from leaking out and relaxing vital muscles, such as the diaphragm, which is crucial for breathing. On a more basic level, the scientists predict that BTS will allow detailed studies of the chemical reactions involving myosin that power muscle contraction and many other cellular functions. Small-molecule inhibitors of motor proteins, such as BTS, usually interfere with cell division. By doing so, they may also lead to new drugs to treat cancer, which at its root is a disease caused by uncontrolled cell division.

Cheung A, Dantzig JA, Hollingsworth S, Baylor SM, Goldman TJ, Mitchison TJ, Straight AF: A Small-Molecule Inhibitor of Skeletal Muscle Myosin II. Nature Cell Biology 4: 83-88, 2001.

## Cells on the Move

*Background*: Cells move around constantly in the body. This movement is critical for normal processes like the development of embryos and the proper functioning of the immune system. But since errant cell movement is a feature of many diseases, scientists are working to understand the fundamental – but very complicated – biology of the movement of cells. For example, the transformation of a stationary cell into an invasive one is a crucial step in metastasis, the movement of cancer cells throughout the body. While scientists know a lot about how cancer cells travel through the lymphatic system, little is known about how cells dislodge from an original tumor and move elsewhere in the body.

*Advance*: Basic researchers using fruit flies as a model system to investigate ovary development have figured out how kickstarting a cell signaling pathway prompts a group of normally stationary cells lining the ovary to travel toward an oocyte (a future egg). Using clever tools of genetics, the scientists discovered how three molecules work together to trigger a cell communication relay called the "JAK-STAT" pathway. Scientists already knew that this signaling pathway plays a role in controlling cell division and cell survival in both flies and humans, and they knew that the relay system is "on" all the time in many cancers. The new work reveals that the JAK-STAT pathway can also convert cells that were "sitting still" into invasive ones that move around.

*Implications*: Understanding how ovarian cells mobilize in fruit flies may help explain how human tumor cells become metastatic. The research is important in revealing a biochemical basis for a poorly understood step in cancer progression. The scientists are now examining ovarian cancer tissue to see if the JAK-STAT pathway can control the movement of human cancer cells.

Silver DL, Montell DJ: Paracrine Signaling Through the JAK-STAT Pathway Activates Invasive Behavior of Ovarian Epithelial Cells in *Drosophila*. Cell 107: 831-841, 2001.

## Gene Silencer Also Controls Development

*Background*: The body's process of reading the genetic code – switching genes on and off at the correct time – is prone to mistakes. Usually, errors are fixed immediately, but sometimes they are not, and genes are turned on at the wrong time. This can cause uncontrolled cell growth and division and lead to diseases like cancer. Basic researchers working with model organisms have identified many of the molecular cogs and gears of the gene regulation process. Scientists know that one molecule in particular, called "Sir2," is an extremely important protein in controlling gene activity. Sir2 helps keep certain genes turned off in inactive regions of DNA, a process known as gene silencing. Researchers have linked faulty gene silencing to leukemia, colon cancer, and some forms of breast cancer.

*Advance*: Now, scientists have discovered a version of the Sir2 gene in fruit flies, a popular model system for genetic research. A team of developmental biologists found that the fruit fly version of Sir2 works similarly to the Sir2 protein in other organisms like yeast and roundworms, silencing genes when their activity is no longer required. However, the fruit fly version of the Sir2 protein appears to be different. The researchers discovered that the protein plays a second important role in flies: helping to account for proper development of the fly's body plan.

*Implications*: In addition to clarifying the function of a molecule that is critical for setting proper levels of gene activity, the new work has practical significance for biomedical researchers investigating the molecular roots of cancer. Finding Sir2 in fruit flies makes this well-studied insect model system useful for the study of cancer genetics in humans. Because some of the key components of Sir2's gene-silencing pathway in yeast, worms, and now fruit flies are identical to those in humans, scientists can exploit these model systems more fully to gain a deeper understanding not only of cancer, but also of embryonic development.

Rosenberg MI, Parkhurst SM: *Drosophila* Sir2 is Required for Heterochromatic Silencing and by Euchromatic Hairy/E(Sp1) bHLH Repressors in Segmentation and Sex Determination. Cell 109: 447-458, 2002.

## Cells That Live and Let Die

*Background*: In the developing nervous system, cells play their own version of television's popular *Survivor* series with a bit of a twist. Rather than being voted off by their teammates, some cells in the developing nervous system will automatically die unless adjacent cells select them to live. Scientists have theorized about how this process works, but until now they had not identified the exact mechanisms. It is an important question because brain cells that fall short of their normal life spans are associated with such devastating diseases as Parkinson's and Alzheimer's. More than half a million Americans have Parkinson's disease,[3] while Alzheimer s disease affects an estimated 4 million Americans.[4] On the other hand, many cancerous tumors result in part from cells' escaping the mechanisms that determine when they should die.

*Advance*: Researchers have for the first time identified an important molecular pathway that nervous system cells use to signal neighboring cells to survive. Without this signal, the cells die. Using fruit fly embryos, the team of cell biology researchers found "trophic factors" in the developing nervous system that certain cells use to direct their neighbors to survive. The scientists also identified how the surviving cells stimulate the development of nerve cells that transmit messages within the nervous system. The researchers studied the development of fruit fly brain cells because they are similar to human brain cells. Their findings could provide a model for studying cellular death and survival in human brains and possibly other parts of the body.

*Implications*: This research may one day lead to new treatments for degenerative neurological diseases, such as Alzheimer's and Parkinson's. These new therapies may keep brain cells alive by providing the necessary "survive" signal. With a greater understanding of cell death and survival, medical researchers also may be able to devise new ways to kill cancer cells. Since one of the traits of cancer is unchecked cell growth, activating the cell death program in cancer cells could halt the disease.

Bergmann A, Tugentman M, Shilo B, Steller H: Regulation of Cell Number by MAPK-Dependent Control of Apoptosis: A Mechanism for Trophic Survival Signaling. Developmental Cell 2: 159-170, 2002.

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[3] *Parkinson s Disease Hope Through Research*, National Institute of Neurological Disorders and Stroke, NIH Publication No. 94-139, September 1994 (updated at http://www.ninds.nih.gov/health_and_medical/pubs/parkinson_disease_htr.htm, July 2001).

[4] *Alzheimer s Disease Fact Sheet*, Alzheimer s Disease Education and Referral Center, NIH Publication No. 01-3431, September 2001.

## Anesthesia Dissected

*Background*: Anesthetics are among the most challenging medicines to administer because they have a wide variety of effects on the body. These drugs not only cause sedation and loss of consciousness, they also produce amnesia (memory loss), analgesia (pain relief), and muscle relaxation (which keeps a patient still during surgery). The complex actions of anesthetics make it difficult for researchers to understand exactly how the drugs work.

*Advance*: A team of anesthesiologists has figured out that anesthetics affect different brain areas to produce two different effects: sedation and memory loss. The scientists measured electrical activity in the brains of healthy volunteers receiving anesthetics, as the people listened to different sounds. To determine how sedated the people were, the researchers measured reaction time to the sounds the people heard. The scientists analyzed memory effects by quizzing the people at the end of the study about word lists they had heard before and during anesthesia. This experimental approach allowed the researchers to measure sedation and memory independently. In combination with imaging studies of brain activity during similar tasks, this study sheds new light on how anesthetics work in different ways in the brain to cause sedation or memory loss.

*Implications*: The study results may help physicians give anesthetic medicines more effectively and safely and prevent reactions with other drugs a patient may be taking. The findings could also point the way to new anesthetics. Future imaging studies should show more precisely which brain regions are affected by anesthesia to produce its many different effects.

Veselis RA, Reinsel RA, Feshchenko VA: Drug-Induced Amnesia is a Separate Phenomenon from Sedation. Anesthesiology 95: 896-907, 2001.

## Surprise Weapon to Treat Unwanted Angiogenesis

*Background*: Angiogenesis is the process in which existing blood vessels branch off to form new ones. The body controls angiogenesis very stringently, and it only occurs under limited circumstances, such as wound healing, pregnancy, and menstruation. Abnormal angiogenesis is involved in many health problems, including cancer and certain kinds of vision loss. Scientists know that many naturally occurring molecules control angiogenesis. There are no FDA-approved treatments to quell unwanted blood vessel growth, but angiogenesis-curbing molecules such as angiostatin and endostatin are currently in clinical testing for the treatment of cancer.

*Advance*: A team of biologists has unearthed a new and entirely unexpected potential therapy for abnormal angiogenesis. The researchers discovered a molecule called T2-TrpRS that can completely block angiogenesis in laboratory mice. Cells make T2-TrpRS by clipping it from its larger, "parent" form (TrpRS). The larger TrpRS protein has no impact on angiogenesis, but the shorter form regulates blood vessel growth.

*Implications*: The discovery of a novel, natural angiogenesis inhibitor showcases the value of untargeted basic research on cells and their proteins. The T2-TrpRS molecule may lead to new treatments for cancer and eye diseases caused by an overgrowth of vessels in the retina, such as a form of age-related macular degeneration and vision loss in people with diabetes.

Wakasugi K, Slike BM, Hood J, Otani A, Ewalt KL, Friedlander M, Cheresh DA, Schimmel P: A Human Aminoacyl-tRNA Synthetase as a Regulator of Angiogenesis. Proc Natl Acad Sci 99(1): 173-177, 2002.

Otani A, Slike BM, Dorrell MI, Hood J, Kinder K, Ewalt KL, Cheresh D, Schimmel P, Friedlander M: A Fragment of Human TrpRS as a Potent Antagonist of Ocular Angiogenesis. Proc Natl Acad Sci 99(1): 178-183, 2002.

## Anthrax Toxin Structure Solved

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This advance grew out of basic research on cell communication that initially appeared to have little immediate significance for human health. It is now directly confronting a critical public health threat. In the words of the biochemist who led the research: "Three years ago, when we started this project, *Bacillus anthracis* was an obscure agricultural pathogen with interesting biological properties. Now, anthrax is front and center in every clinician's mind. ...We hope this work will quickly lead to new therapies."

Drum CL, Yan S-Z, Bard J, Shen Y-Q, Lu D Soelaiman S, Grabarek Z, Bohm A, Tang W-J: Structural Basis for the Activation of Anthax Adenylyl Cyclase Exotoxin by Calmodulin. Nature 415: 396-402, 2002.

## Gene-Environment Interaction Associated with Risk in Maltreated Children

*Background*: Research has shown that maltreated children are at risk for later adjustment problems, including antisocial behavior, conduct disorder, and violence. Although there can be increased risk for later criminality, most maltreated children do not become adolescent or adult violent offenders. The reasons for this variability in response to maltreatment are largely unknown. Recently, however, NIH-sponsored researchers have identified a gene-environment interaction in which variation in the genetic sequence coding a particular brain chemical appears to influence whether or not a maltreated child later develops antisocial behavior.

The Dunedin Multidisciplinary Health and Development Study, a longitudinal study of male children, examined the development and course of antisocial behavior and a range of mental health problems, as well as why some maltreated children grow up to develop antisocial behaviors while others do not. The children, who have been studied from birth to adulthood, completed a comprehensive battery of physical and mental health assessments that tracked behavioral symptoms, neuropsychological functioning, motor development, and cognitive development over time. DNA samples were collected when the participants were 26 years of age.

*Advance*: The researchers found that multiple forms of the gene encoding the enzyme monoamine oxidase A (MAOA), which regulates many neurotransmitters in the brain, moderated the effect of maltreatment. That is, as adults, 85 percent of the severely maltreated children with a form of the gene that conferred *low* levels of MAOA expression were convicted of violent crimes or had other antisocial behavioral outcomes. However, severely maltreated children with a genotype conferring *high* levels of MAOA expression were less likely to develop antisocial behavior problems later in life. Only 12 percent of the entire sample of children had the combination of low MAOA activity level and experiences of maltreatment, yet they accounted for 44 percent of the overall convictions for violent offenses. These findings point to the genotype as a potential moderator of children's sensitivity to environmental risks for antisocial behavior. These results may explain genetic susceptibility or vulnerability to adversity and why some child victims of maltreatment grow up to victimize others and why others do not.

*Implications*: These findings provide preliminary evidence of a gene-environment interaction associated with risk for maladjustment. The results indicate that the one variant of the genotype seems to have a protective effect against the trauma of maltreatment. With appropriate replication of findings, this line of inquiry may help to identify children most vulnerable to adjustment problems resulting from earlier maltreatment and other trauma.

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R: Role of genotype in the cycle of violence in maltreated children. Science 297(2): 851-854, 2002.

## Complex Trait Analysis of Transcriptional Networks

*Background*: Genes are regions of DNA that contain codes for proteins. Proteins are not synthesized directly from the DNA that encodes them, however, but in two sequential steps: the *transcription* of DNA in mRNA, followed by *translation* of mRNA into protein. Understanding the control and modulation of transcriptional networks in the central nervous system (CNS) is now regarded as one of the most critical problems in basic and clinical neuroscience. This is a special challenge for a heterogeneous structure like the brain with hundreds of unique cell types. A group of investigators supported by NIH's multi-institute Human Brain Project has developed a powerful new bioinformatic method to analyze complex transcriptional infrastructure. The method exploits isogenic lines of mice that can be studied using a battery of computational, statistical, molecular, and even morphometric methods. Databases on variation in gene expression are coupled to image and brain behavioral databases. A researcher interested in a particular behavioral phenotype in mice (e.g., anxiety) can search for transcriptional regulators that may influence that trait, thus identifying major causes that underlie the variation. One key method that this group has developed is called transcriptome-QTL mapping. The essential feature of this method involves exploiting statistical gene mapping methods to define the upstream loci (also known as quantitative trait loci or QTLs) that modulate transcriptional activity of large sets of downstream target genes.

*Advance*: In work with animals, the investigators have discovered that variation in expression level of several hundred transcripts maps precisely to the same chromosomal location of the transcript itself. Under study are both so-called *cis*-QTLs, (*cis* indicates that transcription factors are contained on the same stretch of DNA as the genes they regulate) and trans-acting QTLs (meaning the transcript and its controlling QTL do not share a common chromosomal location). The investigators now have successfully mapped over 300 trans-acting QTLs that group into 20 major "master" control QTLs. A single important upstream control QTL appears to be capable of influencing the expression of dozens or even hundreds of downstream targets. In some cases, families of several hundred genes have been shown by the investigators to be controlled by single polymorphic regions of the mouse genome that probably correspond to master control factors. The main issue and challenge now is to extract the common denominator of these families – what is the specific factor and cascade that coregulates these sets of downstream targets?

*Implications*: The transcriptome-QTL method and the results of the first application of this method to the mouse forebrain represent a major step forward in the genetic dissection of transcriptional networks. The integration of this data with preexisting behavioral and anatomical data will provide more rapid progress on CNS disease, behavioral abnormalities, and identification of novel therapeutic targets.

Threadgill DW, Hunter KW, Williams RW: Genetic dissection of complex and quantitative traits: From fantasy to reality via a community effort. Mammalian Genome 13: 175-178, 2002.

## Mind Mapping: Cortical Cartography Takes on New Latitude

*Background*: Elucidating the molecular mechanisms that underlie the development of complex cortical structures is paramount to understanding the evolution of higher brain function. By constructing cortical "maps," on the basis of the function of neurons within a particular structural region, neuroscientists can chart new territory in understanding the workings of the human mind. At the center of this cartographic effort is the use of animal systems to identify molecules and signals that direct mammalian cortical development. To date, however, technological limitations have precluded the precise manipulations required to determine the establishment of functional neural boundaries in the cortex.

*Advance*: Previous characterization of expression patterns of the growth factor FGF8 and its receptors in the anterior portion of the developing brain suggest that this secreted protein is a crucial signal in specifying the boundaries between discrete areas of the cortex. Using a novel technique called "in utero electroporation," NIH-funded researchers now have successfully modified the expression and function of this protein by direct transfer of the FGF8 gene into specific regions of embryonic mouse brain. This elegant procedure allows specific manipulation of gene expression during early, critical developmental periods. Three in utero electroporation manipulations, followed by assessment of cortical boundary "marker" proteins, were performed. Augmentation of FGF8 expression in the anterior cortex resulted in an increase in size of the anterior region and a corresponding posterior shift of parietal and occipital domains of the cortex. Conversely, antagonism of FGF8 signaling resulted in a smaller anterior domain and expansion of posterior cortical regions. Finally, to determine whether FGF8 manipulation merely distorted boundaries or more precisely specified cortical patterning, the researchers engineered ectopic expression of FGF8 in the posterior portion of the brain, and found a role in cortical specification. Taken together, these results support the idea that alterations in the expression and function of a single signaling molecule during development may have profound effects on development of the mammalian cortex, including in areas related to cognition and sensory function in humans.

*Implications*: This landmark study provides the first direct demonstration that a secreted molecule regulates and specifies the formation of the neocortical map. FGF8 and other signaling molecules are expressed in the developing human brain, suggesting that alterations in the expression of or response to secreted factors may be at the basis of a spectrum of disorders involving cortical dysgenesis, including psychiatric disorders of developmental etiology. This crucial technological advance will hasten the identification of additional signals that mediate development of complex cortical structure and function, allowing scientists to create a more complete map of the human mind.

Fukuchi-Shimogori T, Grove EA: Neocortex patterning by the secreted signaling molecule FGF8. Science 294: 1071-1074, 2001.

Rakic P: Neurocreationism – Making new cortical maps. (Perspective) Science 294: 1011-1012, 2001.

## Mouse Model Provides New Insight into Memory Storage

*Background*: Learning and memory involve changes in the strength of connections between nerve cells in the brain. During memory formation, the strength of nerve cell connections (synapses) can either increase or decrease. Strengthening of synapses is referred to as long-term potentiation (LTP), while weakening of synapses is called long-term depression (LTD). Many studies have demonstrated the importance of LTP in memory storage; however, the exact role of LTD, or synaptic weakening, in learning and memory is much less clear. One view is that synaptic weakening has a negative effect on memory. Alternatively, strengthening and weakening of synapses may both be important for optimal memory storage.

There are several different types of learning and memory, which require different skills and presumably have somewhat different underlying biological mechanisms. Short-term (working) memory encodes and uses information from a current, ongoing experience, whereas long-term (reference) memory encodes information learned over a series of experiences. Both working and reference memory occur mainly in a brain region called the hippocampus, where LTP and LTD also take place. However, the relative contributions of LTP and LTD to working memory and reference memory are not well understood.

*Advance*: Using a new mouse model, NIH-funded scientists investigated the involvement of LTD in working and reference memory. The research team used state-of-the-art genetic techniques to delete the gene for calcineurin, a protein necessary for LTD. The mutant mice first stop producing calcineurin at 1-2 months of age (in early adulthood), and adult mice lack calcineurin specifically in the forebrain, which contains the hippocampus. Compared to normal mice, the mutants showed diminished LTD and enhanced LTP in the hippocampus. Furthermore, the mutant mice were specifically impaired in behavioral tests of working memory, but performed normally in tests of reference memory. These results suggest that LTD may be more important for working memory than for reference memory and that weakening of synapses may play an important role in memory storage.

*Implications*: This study reveals a novel difference in the cellular mechanisms that underlie the different types of learning and memory. Moreover, the work supports the view that both strengthening and weakening of nerve cell connections are important for optimal working memory. A better understanding of how the brain accomplishes learning and memory formation may lead to new therapeutic strategies for disorders of learning and memory, such as learning disabilities and Alzheimer's disease.

Zeng H, Chattarji S, Barbarosie M, Rondi-Reig L, Philpot BD, Miyakawa T, Bear MF, Tonegawa S: Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. Cell 107: 617-629, 2001.

## Building an Animal Model of HIV Infection

*Background*: A critical gap in the field of HIV-1 research is the lack of a reliable small-animal model to study how viruses develop, screen new drugs, and test vaccines. Among the problems of existing animal models are limited availability and high cost of nonhuman primates, the absence or delayed progression to an acquired immune deficiency syndrome in some models, or the inability to permit replication of novel retroviruses. Current xenotransplant models (in which cells from one species are injected into another) are informative about select aspects of HIV-1 pathogenesis, but present neither a complete range of infected tissues nor the context of an intact immune response.

*Advance*: An NIH-supported researcher has been attempting to fill this gap in the HIV research field by developing a rat model for HIV neuropathogenesis studies. The rat has several advantages as a potential small-animal model, including size, convenience of breeding, and a well-characterized immune and central nervous system. In addition, transgenic rats can be generated with relative ease, enabling the selective expression of human genes that may be essential for realizing fully the HIV-1 replication cycle. The researcher's initial studies focused on defining the capacity of HIV-1 to replicate in rat-derived cells in vitro, and extensively characterized factors that either permitted or prohibited rat-derived cells to allow for HIV-1 replication. This work set the stage for developing a transgenic rat model to study how HIV impairs nerve cells.

The investigator next embarked on in vivo studies, culminating in the development of a rat model that expresses human CD4 and human chemokine receptor molecules, which are required for viral entry into cells. Cells derived from these rats were highly susceptible to infection by HIV-1, and viral replication was comparable to that found in human cells. HIV-1 susceptibility was also demonstrated in vivo. The rats displayed low-level plasma viruses early in infection. As a result, rats that express the appropriate human receptor complexes are promising candidates as a small-animal model for HIV-1 infection.

*Implications*: The availability of a small-animal model for HIV/AIDS research offers a unique opportunity to study mechanisms of HIV neuropathogenesis that are difficult to study in humans or nonhuman primates. In addition, this model has enormous potential for use in testing novel drug candidates and vaccines.

Keppler OT, Yonemoto Y, Welte FJ, Patton KS, Iacovides D, Atchison RE, Ngo T, Hirschberg DL, Speck RF, Goldsmith MA: Susceptibility of rat derived cells to replication by HIV Type 1. J Virol 75(17): 8063-8073, 2001.

Keppler OT, Welte FJ, Ngo, TA, Chin PS, Patton KS, Tsou CL, Abbey NW, Sharkey ME, Grant EM, You Y, Scarborough JD, Ellmeier W, Littman DR, Stevenson M, Charo IF, Herndier BG, Speck RF, Goldsmith MA: Progress toward a human CD4/CCR5 transgenic rat model for de novo infection of human immunodeficiency virus Type 1. J Exp Med 195: 719-736, 2002.

## Olfactory Neurons as a "Window" on the Etiology of Schizophrenia

*Background*: Schizophrenia is a severe mental illness characterized by disordered thinking and mood, impaired social interactions, delusions, hallucinations and psychosis. It affects about 1 percent of the population. Research suggests that schizophrenia is caused by biological abnormalities in multiple brain systems and that these abnormalities probably are initiated while the brain is developing. Because so much remains unknown about how brain cells develop in normal humans (much less in those destined to develop schizophrenia), the neurodevelopmental defect(s) that may underlie schizophrenia continue to elude scientists. The discovery that limited neural development occurs in adults provides a potential opportunity for seeing how developing brains cells might differ between individuals with and without schizophrenia. However, stem cell research is relatively new, and a great deal about normal adult stem cell development remains to be elucidated. Fortuitously, there is one other specialized population of neural cells that continues to develop throughout the life span and about which a significant amount is known – olfactory neurons. What's more, patients with schizophrenia, as well as their relatives who do not have schizophrenia, have numerous characteristic defects in their olfactory systems, making this a likely place to look for abnormalities.

*Advance*: Putting these facts together, NIH-supported researchers have developed an innovative approach to the question of whether neurodevelopment is anomalous in schizophrenia. Using antibody stains specific for three different stages of maturation in olfactory neurons (basal or "stem" cells, immature neurons, and mature neurons), the investigators examined autopsied brains from patients with schizophrenia and from unaffected control subjects. The number of mature cells did not differ between the patients and the controls. However, the number of olfactory stem cells was lower and the number of immature neurons was higher in the patients, even when smoking and medication history were controlled for. Apparently the development of these neurons is first accelerated, thereby depleting the stem cell population, and then arrested at an immature stage in the brains of patients with schizophrenia.

*Implications*: The authors point out that a similar pattern has been seen in animals when olfactory cells are prevented from making their proper connections with other neurons, and this problem may be characteristic of brain development in other areas that are known to be abnormal in individuals who develop schizophrenia. This provides a testable paradigm with which to explore the hypothesis that schizophrenia is a neurodevelopmental disorder.

Arnold SE, Han LY, Moberg PJ, Turetsky BI, Gur RE, Trojanowski JQ, Hahn CG: Dysregulation of olfactory receptor neuron lineage in schizophrenia. Arch Genl Psychiatry 58: 829-835, 2001.

## Narrowing the Window of Fetal Vulnerability for Adult Schizophrenia

*Background*: Recent findings from psychiatric epidemiological studies suggest that some forms of schizophrenia may result from disruption of normal brain development during the sixth month of gestation. Further specifying the window of fetal vulnerability may help pinpoint the brain areas and circuitry related to the etiology of schizophrenia. One strategy for discerning critical risk periods is to study individuals exposed to agents that cause disruption at different stages of prenatal neural development.

*Advance*: NIH researchers assessed schizophrenia-related features in 2,309 young men who were and 2,065 who were not exposed in utero to an influenza epidemic. Schizotypal personality characteristics, as measured by scores on the Minnesota Multiphasic Personality Inventory Schizophrenia and Psychasthenia (disorder characterized by phobias, obsessions, compulsions, or excessive anxiety) scales at age 20, were compared for the exposed and non-exposed (control) subjects. The study was designed to identify discrete periods of neural development associated with onset of the disorder in adulthood. A significantly higher proportion of subjects exposed in utero to an influenza epidemic during the sixth month of pregnancy (39 percent) had elevated schizotypal personality scale scores (i.e., upper quartile) compared to the non-exposed (26 percent). These differences were accounted for by exposure during gestation week 23; subjects exposed during week 23 (index group) were more likely to achieve elevated schizotypal personality scale scores (51 percent) than their controls (24 percent). Exploratory analyses for the other months revealed no significant differences between the index and control groups in vulnerability to schizotypal personality disturbance.

*Implications*: This study builds on prior research pointing to the second trimester – particularly the sixth month – as a critical period in the genesis of schizophrenia vulnerability. The results further refine the window of vulnerability to the 23$^{rd}$ week of gestation, and suggest new hypotheses regarding neuromaturational events that may be related to the etiology of schizophrenia.

Machon RA, Huttunen,MO, Mednick, SA, Sinivuo,J, Tanskanen A, Watson JB, Henriksson M, Pyhala R: Adult schizotypal personality characteristics and prenatal influenza in a Finnish birth cohort. Schizophrenia Research 54: 7-16, 2002.

## Face Processing in Autism Engages Unusual Neural Circuitry

*Background*: A major feature of autism is the lack of social interaction. Studies of face processing, or recognition, using functional magnetic resonance imaging (fMRI) have consistently demonstrated thata specific region of the fusiform gyrus ("the fusiform face area") is active when healthy individuals view faces. This region may be genetically programmed for processing faces or, alternatively, may subserve visual recognition of objects in which people have abundant experience and expertise.

Most people become experts at recognizing familiar faces in the course of normal development; people with autism do not. Whereas typically developing infants attend vigorously to faces, this predisposition is disrupted in autism. Young children with autism may make little eye contact and fail to recognize others based on facial features, instead relying on nonessential and changeable details such as hair style, glasses, or hats that, understandably, may lead to failures of recognition. These developmental disruptions may be early events in a cascade of missteps leading to profound social impairments. Moreover, while typically developing children are better able to process faces when presented in an upright, as opposed to inverted, orientation, those with autism process inverted faces as well as upright faces, perhaps because they rely on individual details, rather than processing faces in a holistic fashion. Thus, people with autism appear to process faces in a manner that differs qualitatively from those of their healthy peers.

*Advance*: NIH-sponsored researchers recently investigated the neural correlates of face processing in autism. Seven high-functioning adults with autism and eight normal controls performed a face perception task (pressing a button in response to female faces) and a control task (shape perception, pressing a button in response to circles), while changes in cerebral blood flow and volume (an indirect indication of neuronal activity) were measured with fMRI. Both groups were able to perform the tasks well, with no significant differences seen in accuracy or speed. Consistent with earlier research, healthy control subjects activated the fusiform face area, particularly on the right side of the brain. In contrast, subjects with autism showed greatly reduced activation of this region (on average, 25 percent of that seen in controls). In addition, controls, but not patients, activated the amygdala, a limbic structure thought to be involved in assigning social-emotional significance to objects, including faces. The fusiform face area showed the greatest activation in every control subject. In contrast, individual subjects with autism maximally activated other brain regions, which differed from person to person, suggesting that individuals with autism see faces using uniquely programmed neural circuitry.

*Implications*: This work may suggest that developmental experiences, which themselves may be influenced by early brain abnormalities, play a critical role in specifying the neural sites that respond to human faces in autism. This suggests the more general proposition that neurodevelopmental abnormalities that lead to the establishment of inefficient and/or faulty neural networks may play a causative role in autism.

Pierce K, Muller R-A, Ambrose J, Allen G, Courchesne E: Face processing occurs outside the fusiform "face area" in autism: Evidence from functional MRI. Brain 124: 2059-2073, 2001.

## Understanding the Molecular Basis of Antipsychotic Drug-Induced Weight Gain in Schizophrenia

*Background*: Although new antipsychotic drugs (APDs) have been introduced that improve negative symptoms and cognitive deficits in schizophrenia, short-term and long-term weight gain are an unwanted side effect. The adverse consequences of APD-induced weight gain are an increased risk of cardiovascular disease and type II diabetes. The magnitude of weight gain and its attendant increases in morbidity and mortality can diminish the positive effects of APDs that produce this effect to the greatest extent (e.g., clozapine and olanzapine) and lead to treatment noncompliance and serious medical complications.

*Advance*: Two recent studies have shed light on the molecular mechanisms underlying APD-induced weight gain, and both studies implicate the involvement of chemically specified neurons in hypothalamic areas known to be involved in control of appetite and food intake. In the first study investigators measured Fos protein, a marker of neuronal activity, to assess the effects of APDs on the activity of orexin neurons in the hypothalamus. They found that APDs that significantly increase weight gain [clozapine (Clozaril), olanzapine (Zyprexa), risperidone (Risperdal), and chlorpromazine (Thorazine)] produced a large increase in Fos protein, whereas APDs with low weight gain liability [haloperidol (Haldol), fluphenazine (Permitil, Prolixin), and ziprasidone (Zeldox)] did not. These findings suggest that orexin neurons in the hypothalamus may be involved in APD-induced weight gain. Future studies will determine which transmitter receptors are involved in regulating the activity of orexin neurons.

In a second study, investigators examined the receptor binding profile of atypical and typical APDs to determine which receptors are contributing to APD-induced weight gain. Using statistical analysis to determine the relationship between receptor binding and weight gain, they found a striking correlation for the ability of APDs to bind to the H1 histamine receptor and their propensity to induce significant weight gain. The APDs with high affinity for the H1 histamine receptor were associated with significant weight gain [the atypical APDs clozapine, olanzapine, risperidone, sertindole, quetiapine; and the typical APDs chlorpromazine, thiothixene, perphenazine], whereas the APDs with low affinity for the H1 receptor were not [the atypical APDs ziprasidone, aripiprazole; and the typical APDs haloperidol, fluphenazine, trifluperazine, pimozide].

*Implications*: Together, these studies significantly advance our understanding of the molecular basis of APD-induced weight gain. In addition, these studies suggest that the development of APDs with low or no affinity for H1 receptors will lead to a new generation of APDs with a more favorable side effect profile.

Fadel J, Bubser M, Deutch A: Differential activation of orexin neurons by antipsychotic drugs associated with weight gain. J Neurosci 22: 6742-6746, 2002.

Kroeze WK, Hufeisen SJ, Popadak BA, Rennock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Roth BL: H1-Histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. Neuropsychopharmacology (in press).

## Evaluating Threat in Social Situations: The Role of the Amygdala

*Background*: The amygdala is a key brain area posited to be centrally involved in emotions. To understand its precise role, NIH-sponsored investigators developed a technique to functionally eliminate the amygdala without damaging neural fibers that pass through that area carrying important information. When the amygdala was eliminated in adult monkeys, they were seen to be capable of interpreting and generating social gestures from other monkeys and to be able to solicit affiliative social interactions from others, more so than normal animals. Thus, contrary to a commonly held view, the amygdala appeared not to be necessary for carrying out social behaviors. But is the amygdala involved in *learning* appropriate social behaviors?

*Advance*: To answer this question, the same research team compared monkeys from which the amygdala was removed at two weeks of age to normal infant animals. At two weeks, infant monkeys typically cling to their mothers' sides, and there is virtually no social interaction with other monkeys. The interactions between infants and their mothers were similar in animals with lesioned amygdalas compared to normal animals. Like the adult animals, the infants with lesioned amygdalas showed little fear of objects (like rubber snakes) that normally provoked fearful behavior; in novel interactions with other monkeys, however, they showed increased fear and less interaction. In light of the absence of the amygdala, the investigators deduced that social fear must be mediated by a different brain region. Fear reaction in the context of dyadic interactions notwithstanding, the lesioned animals showed substantial social behavior that seemed similar to normal animals of the same age (grooming, play, facial expressions).

*Implications*: The evidence has led to the hypothesis that a primary role of the amygdala is to evaluate the environment for potential threat, a role that has been explored extensively in relation to conditioned fear or startle responses. In studies by these investigators, where the focus has been on social behavior, amygdala lesions seem to have produced socially "uninhibited" animals whose normal reluctance to engage a novel animal appears to have been eliminated. That is, without a functioning amygdala, macaques do not evaluate monkeys unknown to them as potential adversaries. The amygdala does not appear to be essential for either learning social behavior at an early age, or for the production of social behavior as adults. Rather, the amygdala may modulate the amount of social behavior based on an evaluation of the safety of the social context. The research encourages speculation that there may be a form of social anxiety disorders in humans that includes a dysregulation of the amygdala's evaluative process.

Prather MD, Lavenex P, Mauldin-Jourdain ML, Mason WA, Capitanio JP, Mendoza SP, Amaral DG: Increased social fear and decreased fear of objects in monkeys with neonatal amygdala lesions. Neurosci 106(4): 653-658, 2001.

## Brain Circuitry Underlying Addiction, Obsessive-Compulsive Disorder

*Background*: During any task, we continually compare our current status against our expectation for reaching a goal, with expectation increasing over the course of the activity. Presumably, there are neurons in the brain that signal this rising expectation for completion. One obvious place to look for such signals is the anterior cingulate cortex, a region deep in the front of the brain that has been shown to contribute to generation of emotions, and the influence of emotions on behavior selection.

*Advance*: Researchers at NIH have looked for such signals in single neurons of the brains of non-human primates. They trained the monkeys to perform a repetitive task; if the monkey completed enough repetitions correctly, it received its favorite fruit juice as a reward. As the reward neared, the monkeys worked more accurately, as though driven to complete the task. The investigators found a group of neurons in the anterior cingulate cortex whose activity increased in parallel with the reward expectancy. The activity abated when the goal was achieved and the state of anticipation resolved.

*Implications*: Abnormalities of the anterior cingulate cortex have been implicated in a variety of behavioral disorders including obsessive-compulsive disorder, post-traumatic stress disorder, depression, and mania. Understanding the brain circuitry underlying the role of emotions in behavior will allow development of more effective behavioral therapies for these disorders.

Shidara M, Richmond BJ: Anterior cingulate: Single neuronal signals related to degree of reward expectancy. Science 296: 1709-1711, 2002.

## Novel Brain Mapping Reveals How Genes Affect Human Brain Structure

*Background*: Despite large-scale efforts to map genetic and brain variations in human populations, there have been no technologies available to link these two types of information. In response to this challenge, an international team of scientists designed a brain mapping strategy to create the first maps of genetic influences on human brain structure.

*Advance*: Using a database of MRI scans from the Finnish twin registry, the scientists developed a novel supercomputing approach to encode how brain structure varied between identical and fraternal pairs of twins. Qualities under genetic control show a characteristic pattern of varying hardly at all between identical twins, who have the same genes; quite a lot between fraternal twins, who share about half their genes; and a great deal between unrelated individuals. High-performance computing technology was developed to build three-dimensional maps of each subject's brain, and then to color code the degree of heritability for each brain region. The quantity of gray matter in the frontal lobes was under particularly tight genetic control, and was linked with individual intelligence (IQ), as was a region at the side of the left hemisphere known as Wernicke's area, which is central to language. The study showed that the more closely related two people are, the more likely they are to share similar brain structure in regions heavily controlled by genetics, such as the frontal cortex and language regions. They are also more likely to share vulnerabilities to specific diseases affecting these areas.

*Implications*: This is the first study to create maps showing how strongly brain structure is determined by genes and inheritance. It also provides a technology to examine how genes affect brain structure in thousands of subjects. The mapping strategy is now being used to attempt to identify genetic and non-genetic triggers in diseases such as schizophrenia and dementia. Finding which structures are under greatest genetic control provides clues to where to look for degeneration in family members at risk for disease. The new maps pinpoint the areas at risk, allowing the team to examine differences in genetic profiles among family members, some of whom have a language or frontal cortex disorder – including schizophrenia and frontotemporal dementia – and others who do not. The scientists are applying the new brain mapping method in several international projects to screen individuals at genetic risk for schizophrenia and Alzheimer's disease for early brain changes.

Thompson PM, Cannon TD, Narr KL, van Erp TGM, Poutanen VP, Huttunen M, Lönnqvist J, Standertskjöld-Nordenstam CG, Kaprio J, Khaledy M, Dail R, Zoumalan CI, Toga AW: Genetic influences on brain structure. Nat Neurosci 4(12): 1253-1258, 2001.

Thompson PM, Cannon TD, Toga AW: Mapping genetic influences on human brain structure. Annals of Medicine 34: 1-14, 2002.

## Amygdala Response to Fearful Faces

*Background*: NIH researchers previously identified a region of genetic variability in humans in the gene that codes for the serotonin transporter. This protein and its gene are important to psychiatrists because the newest class of antidepressants, the selective serotonin reuptake inhibitors (SSRIs, including Prozac), are believed to act on this protein in the brain. People who have the "short" variant of the gene have less of the protein in their brain and are somewhat more likely to develop depression or display abnormal levels of anxiety. Approximately 40-60 percent of the population has the "short" version of the gene.

Researchers who are interested in the brain circuitry of emotion in humans have frequently used functional magnetic resonance imaging (fMRI) methods to examine brain responses to emotional stimuli. These sorts of studies have centered on a structure deep in the brain, the amygdala, which is known to be involved in emotional behaviors in other species. They have found that the amygdala is activated when people see a face with a fearful expression, as compared to when they see a face with a neutral or happy expression.

*Advance*: Now NIH researchers have brought together these two lines of research. They used fMRI to compare the activity in the amygdala of people who have the "short" version of the serotonin transporter gene with that of people who have the "long" version. They found that those with the "short" version have more activity in their amygdala when they see the fearful faces. This difference is much more striking than that seen in previous studies, which looked at more subjective measures. It is important to note that none of the subjects in this study had an affective or other psychiatric disorder. This variation is found within the normal population.

*Implications*: Identifying genes involved in behavior is only the first step in understanding these processes. Investigators need to determine how particular genes and the proteins they encode act in the brain to affect behavior. This study moves the research field ahead by providing a direct link between a gene difference and a difference in brain activity that is more easily measured than differences in behavior.

Hariri AR, Mattay VS, Kolachana B, Tessitore A, Fera F, Goldman D, Egan MF, Weinberger DR: Serotonin transporter genetic variation and the response of the human amygdala. Science 297: 400-403, 2002.

## Pathophysiology of Auditory Hallucinations

*Background*: Hallucinations are among the most dramatic, disabling, and conspicuous symptoms of schizophrenia. Their neuropathology is poorly understood, but because schizophrenic hallucinations are most frequently auditory, researchers have focused on the part of the brain that "hears" (auditory cortex). One theory about hallucinations is that they may simply be the patient's own thoughts that are misperceived as real words being spoken by someone else rather than as their own internal ruminations. It has been suggested that in healthy individuals a "corollary discharge" occurs somewhere in the brain to signal the auditory cortex that the speech it perceives is coming from the self, rather than from someone outside. If this signal fails to occur when the person is speaking or thinking, the person "hallucinates" that they are hearing a voice.

*Advance*: NIH-funded investigators now have localized the part of the brain that may be responsible for this corollary discharge. Using the N100, an electrical signal generated by the auditory cortex 100 msec after a sound is heard, scientists compared brain activity in patients with schizophrenia and in control subjects. First, they found that in control subjects the N100 signal was dampened when the subject was talking. No dampening effect was seen in the patients. Second, when subjects were instructed to repeat a given sentence silently "in their head," the N100 signal was again dampened in the control subjects but not in the patients. Because it was possible that the control subjects were simply better at paying attention to the task than patients, the investigators verified that the differences only occurred while the subject was talking (aloud or silently), but not while they were listening to speech or noise. Finally, the investigators ascertained that one specific area of the brain – the lower left frontal lobe – was attempting to correlate the electrical signals they were recording all over the subject's head (coherence analysis) in order to see if any region of the brain was consistently activated along with the auditory cortex in the controls, but not in the patients when the subjects were speaking; only a trace of this pattern was found in patients with schizophrenia who did not have hallucinations and it did not exist at all in those patients who did have hallucinations.

*Implications*: The investigators believe that the lower left frontal lobe, which has been shown in the past to be involved in initiating activities (like speech), may be preparing the auditory cortex to recognize that the speech it is going to hear is being self-generated. When this preparation fails to occur, hallucinations result. When the researchers more accurately pinpoint the brain region responsible for this corollary discharge, it may be possible to develop medical or surgical treatments to enhance the missing signal and alleviate hallucinations.

Ford JM, Mathalon DH, Whitfield S, Faustman WO, Roth WT: Reduced communication between frontal and temporal lobes during talking in schizophrenia. Biol Psychiatry 51(6): 485-492, 2002.

Ford JM, Mathalon DH, Heinks T, Kalba S, Faustman WO, Roth WT: Neurophysiological evidence of corollary discharge dysfunction in schizophrenia. Am J Psychiatry 158(12): 2069-2071, 2001.

Ford JM, Mathalon DH, Kalba S, Whitfield S, Faustman WO, Roth WT: Cortical responsiveness during inner speech in schizophrenia: an event-related potential study. Am J Psychiatry 158(11): 1914-1916, 2001.

## Hypertension

*Background*: Hypertension, blood pressure measuring 140/90 mmHg or higher over time, is a serious health problem. If left untreated, it can lead to stroke, heart disease, kidney failure and other health problems. Recent research has led to new insights into the relationship between salt consumption and blood pressure and an understanding of the risk factors for hypertension.

*Advance:* Over the years, researchers have broadly defined the impact of "salt sensitivity," as the blood pressure response to changes in sodium intake among individuals in a population. Provisional answers to this question have emerged based on newly discovered links between sodium metabolism and the generation of reactive oxygen species (ROS). Both "high" and "low" sodium consumption may accelerate cardiovascular aging in different subsets of the population by activating pathways that increase ROS levels and the cumulative burden of oxidative end products.

*Implications:* The relationships between salt consumption and blood pressure no longer predominate because compelling evidence now points to a biological effect of salt mediated by mechanisms that are independent of blood pressure.

Aviv, A: Salt consumption, reactive oxygen species and cardiovascular aging: a hypothetical link. Journal of Hypertension 20 (4): 555-559, 2002.

## Molecular Profiles Predict Post-Chemotherapy Survival Rates for B-cell Lymphoma Patients

*Background*: Diffuse large B-cell lymphoma is the most common adult lymphoma, diagnosed in more than 16,000 new patients each year in the U.S. Only about 40 percent of patients with the disease can be cured by conventional multi-agent chemotherapy regimens. The gap between those who can be cured and those who cannot suggested the possibility that the disease consisted of molecularly distinct subgroups.

*Advance*: This hypothesis was confirmed by a gene-expression profiling study of 240 patient biopsy samples, which differed dramatically in the expression of more than 600 genes. One set of variably expressed genes was used to define two disease subgroups that seem to arise from different stages of normal B-lymphocyte development. Patients with each type had significantly different clinical outcomes, suggesting the subgroups are distinct diseases that are microscopically indistinguishable.

Many other genes also influenced patient survival, and reflected different biological features of the lymphomas that dictated responsiveness to chemotherapy, including the tumor cell proliferation rate and the immune response to tumors. To help predict survival, a molecular composite of 17 key genes was developed; using this tool, patients were divided into four equal groups with 5-year survival rates of 73 percent, 71 percent, 36 percent, and 15 percent.

*Implications:* The molecular outcome predictor may be useful in clinically managing patients with diffuse large B-cell lymphoma; it can identify patients who are likely to be cured by current chemotherapy regimens, including those with aggressive or advanced disease. Patients in the least favorable risk group might benefit from alternative therapies such as bone marrow transplants or clinical trials of novel therapeutic agents.

Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne R, Muller-Hermelink HK, Smeland EB, Staudt LM: The use of molecular profiling to predict survival after chemotherapy for diffuse large B-cell lymphoma. N Eng J Med 346(25): 1937-1947, 2002.

## Association of HLA and KIR Genes with AIDS Progression

*Background*: Researchers have been studying genetic variations that predispose a person to progress from HIV to full-blown AIDS. *HLA* genes help the immune system fight viruses and other foreign substances, but it has been known for some time that a subgroup of *HLA* genes called *HLA-B*35* is associated with rapid progression to AIDS. Certain *KIR* genes can activate immune system natural killer (NK) cells, which have a key role in immune response. Both *HLA* and *KIR* tend to be widely varied in form (polymorphic) throughout the population.

*Advance*: Two studies were conducted. In the first, researchers studied genetic variations of the *HLA* subtype *HLA-B*35* in a group of 850 HIV-1 positive patients. They divided the subtype into two groups, the *HLA-B*35-Px* group and the *HLA-B*35-Py* group, based on peptide-binding specificity. Patients who carried the *HLA-B*35-Px* subtype quickly progressed to AIDS while those with the *HLA-B*35-Py* subtype did not. The difference was significant. This finding was true for both black and white patients in the study. A single amino acid difference between the two *HLA* subtypes was responsible for accelerating the progression to AIDS.

In the second study, *KIR* genotyping was performed in patients from the same HIV-1 positive patient groups (cohorts). One activating *KIR* gene, designated *KIR3DS1*, in combination with a particular *HLA* binding molecule (HLA-Bw4) resulted in delayed progression to AIDS. Conversely, in the absence of HLA-BW4, *KIR3dS1* was significantly associated with more rapid progression to AIDS. The results suggest a synergistic interaction between these *HLA* and *KIR* genes.

*Implications*: This new knowledge should help researchers identify individuals at particular risk for rapid progression to AIDS. Genetic tests may be developed to identify those who carry the *HLA-B*35-Px* subtype and specific *KIR* genes. Physicians will then have the information needed to begin a more aggressive form of treatment in these individuals, particularly those newly diagnosed. These discoveries also provide a deeper understanding of the mechanisms involved in HIV-1 disease and may prove useful in developing effective HIV therapeutics and vaccines.

Gao X, Nelson GW, Karacki P, Martin MP, Phair J, Kaslow R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, O'Brien SJ, Carrington M: Effect of a single amino acid change in MHC Class I molecules on the rate of progression to AIDS. N Eng J Med 344(22): 1668-1675, 2001.

Martin MP, Gao X, Lee J-H, Nelson, GW, Detels, R, Goedert JJ, Buchbinder S, Hoots, K, Vlahov, D, Trowsdale, J, Wilson, M, O'Brien, SJ, Carrington, M: Epistatic interaction between KIR3DS1 and HLA–B delays the progression to AIDS. Nat Gen 31: 429-434, 2001.

## Low Oxygen Levels Activate Kaposi's Sarcoma–Associated Herpesvirus (KSHV)

*Background*: Kaposi's sarcoma (KS) is the most common cancer in people with acquired immunodeficiency syndrome (AIDS). The disease, caused by a virus known as Kaposi's sarcoma-associated herpesvirus, or KSHV (also called human herpesvirus 8, or HHV-8), commonly develops on the feet. The virus can lie dormant in cells for long periods until it is activated. Until now, the stimulus that activates KSHV was unknown.

*Advance*: Researchers discovered that KSHV is activated by a lack of oxygen (hypoxia). Two main findings support this conclusion: (1) KSHV-infected cells exposed to low levels of oxygen have increased production of interleukin-6, which promotes the growth of KS cells, and (2) two chemicals that mimic the effects of hypoxia also induce the replication of KSHV.

*Implications*: This research may lead to treatment strategies for KS. It also helps to explain two observations about Kaposi's sarcoma. First, the feet often have lower tissue oxygen levels than the rest of the body, thus it is logical that Kaposi's sarcoma often appears on the feet. Second, KS is also strongly associated with malaria; until now, this association could not be explained. People with malaria often have very low levels of red blood cells. As a result, they have low oxygen levels in their tissues; this could predispose them to KS.

Davis DA, Rinderknecht AS, Zoeteweij JP, Aoki Y, Read-Connole EL, Tosato G, Blauvelt A, Yarchoan R: Hypoxia induces lytic replications of Kaposi's sarcoma-associated herpesvirus. Blood 97(10): 3244-3250, 2001.

## Mouse Model Provides Experimental Validation of a Critical Role for Childhood Sunburn in Melanoma Development

*Background*: Exposure to the sun's ultraviolet (UV) rays may be responsible for up to 80 percent of malignant melanoma, the most dangerous form of skin cancer. While other types of skin cancer seem to be associated with cumulative sun exposure, melanoma risk appears to increase after intense intermittent UV exposures, especially during childhood. Animal models for skin cancer development have been difficult to develop; mouse melanocytes (the cells that become cancerous in melanoma) are found only in mouse hair follicles, while in humans they are found in all layers of the skin.

*Advance*: NIH-supported researchers used genetically engineered mice with melanocytes in multiple skin layers, more closely resembling human skin. The mice are also genetically predisposed to melanoma. Mice were exposed to UV radiation at 3.5 days of age, 6 weeks of age, or both. In the 3.5 day old group, the single dose of UV radiation led to melanoma; this dose was comparable to the amount of midsummer natural sunlight that would sunburn a human. In the 6-week old group, the UV dose did not lead to melanoma, but did increase the incidence of other types of skin cancer. The group receiving two doses of UV radiation developed melanoma at rates similar to the 3.5 day old group.

*Implications*: This mouse model could prove extremely useful for studying melanoma and its risk factors, both genetic and environmental. The findings suggest that childhood sunburn poses a significant melanoma risk, but these results should not yet be extrapolated to children because (1) the mice in this study were genetically "primed" to develop skin cancer; (2) the difference in the thickness of human skin compared to mouse skin could affect the penetration of UV light, and therefore cancer risk; and (3) the human equivalent of a 3.5 day old mouse is not known.

Noonan FP, Recio JA, Takayama H, Duray P, Anver MR, Rush WL, DeFabo EC, Merlino G: Neonatal sunburn and melanoma in mice. Nature 413: 271-272, 2001.

## Annual Report to the Nation Helps Identify Needs in Cancer Research and Care

*Background*: High quality cancer surveillance is needed in order to help provide direction to cancer research and care so that the needs of all populations will be met. NIH, along with many partners, has been improving surveillance of cancer trends and the use of that data in planning and setting priorities.

*Advance*: *The Annual Report to the Nation on the Status of Cancer, 1973-1999, Featuring Implications of Age and Aging on U.S. Cancer Burden*, was recently released by a number of collaborators: NIH, the Centers for Disease Control and Prevention, the National Center for Health Statistics, the American Cancer Society, and the North American Association of Central Cancer Registries. Researchers noted a decrease in overall cancer death rates from 1993 to 1999, while rates of cancer incidence stabilized over a similar time frame. However, investigators found that individual trends of various populations groups differed substantially, e.g. by cancer site, sex, and race. For example, cancer incidence rates decreased for men under 50 years, but not for men older than 50. Even among men less than 50, incidence of some cancers (prostate, for example), actually increased. For women overall cancer incidence increased, although with variability in the magnitude of the change in different age groups and for different disease sites. For example, breast cancer rates increased for women aged 50-64 and lung cancer rates increased for women 64-70, but decreased in women under age 50. Overall, lung cancer was the leading cause of cancer deaths, followed by colorectal, and then breast and prostate cancer.

*Implications*: Due to the increasing size of the population and the growing proportion of elderly, the cancer burden of the United States is expected to increase substantially over the next several decades – even if death rates continue to decline and incidence rates remain stable. Clearly, the need for better focus on cancer control to serve the aging population is critical. The changing age structure in the United States must direct strategies for cancer prevention and early detection, social support, treatment and medical care, clinical trial design and enrollment, research, and surveillance. In addition, access to supportive, palliative, and general medical services must be optimized.

Edwards BK, Howe HL, Ries LAG, Thun MJ, Rosenberg HM, Yancik R, Wingo PA, Jemal A, Feigal EG: Annual report to the nation on the status of cancer, 1973-1999, featuring implications of age and aging on the U.S. cancer burden. Cancer 94(10): 2766-2792, 2002.

## Inherited *BRCA2* Mutations Increase Risk for Pancreatic Cancer

*Background*: Scientists believe that 10 percent of all cases of pancreatic cancer are hereditary. However, although mutations have been found in the tumors of patients with non-hereditary pancreatic cancers, the major gene(s) responsible for inherited cases have yet to be discovered. Uncovering the causal mutations would provide a way to identify people with an increased genetic risk of developing this disease who might benefit from special surveillance.

*Advance*: NIH Special Program of Research Excellence (SPORE) investigators examined the blood of pancreatic cancer patients who had at least three family members with the disease. The researchers were looking for harmful mutations in four separate genes suspected to play a causal role in this disease. Their study of three of the genes revealed no such mutations. However, they did discover mutations, likely to be harmful, in the *BRCA2* genes of 5 out of 29 (or 17 percent) of patients in the study.

*Implications*: Further research is needed to better define the risk of pancreatic, and other cancers, in patients with these mutations. There are probably additional genetic as well as environmental factors that influence this risk. However, mutations to the *BRCA2* gene appear associated with a relatively high proportion of pancreatic cancers, both inherited and non-inherited.

Murphy KM, Brune KA, Griffin C, Sollenberger JE, Petersen GM, Bansal R, Hruban RH, Kern SE: Evaluation of Candidate Genes *MAP2K4*, *MADH4*, *ACVR1B*, and *BRCA2* in Familial Pancreatic Cancer: Deleterious *BRCA2* Mutations in 17%. Cancer Res 62: 3789-3793, 2002.

Schutte M, da Costa LT, Hahn SA, Moskaluk C, Hoque ATMS, Rozenblum E, Weinstein CL, Bittner M, Meltzer PS, Trent JM, Yeo CJ, Hruban RH, Kern SE: Identification by Representational Difference Analysis of a Homozygous Deletion in Pancreatic Carcinoma that Lies within the BRCA2 Region. PNAS 92: 5950-5954, 1995.

## Exploring the Tumor Microenvironments of Pancreatic and Breast Cancers

*Background*: When healthy tissues of the body are invaded with cancer cells, they become inflamed and form fibrous, scar-like tissue known as "desmoplastic tissue." Infiltrating tumors, those that penetrate surrounding tissues as they grow, usually contain more desmoplasmic tissue than they do cancer cells. Researchers are discovering that unique molecular interactions occur between cancer cells and this type of "tumor microenvironment" (i.e. surrounding tissues). If scientists can characterize these tumor-microenvironment interactions, they may also be able to develop original interventions for cancer prevention, detection, and treatment. NIH Special Program of Research Excellence (SPORE) researchers recently developed a technology, "serial analysis of gene expression" (SAGE), that can perform this characterization.

*Advance*: NIH SPORE investigators used SAGE to study the patterns of genes expressed in the cells, and surrounding tissues, of two types of infiltrating cancer – pancreatic and infiltrating breast cancer. These researchers showed that both types of tumors were made up of four distinct kinds of tissue that reflect a highly ordered, coordinated process of tumor invasion, which involves structured molecular communication between the tumor and its microenvironment.

*Implications*: This work provides new insight into the host response to invasive cancer. Further studies may reveal molecular targets for clinical imaging, diagnosis by blood test, and drug development and delivery for pancreatic, infiltrative breast, and other cancers.

Iacobuzio-Donahue CA, Ryu B, Hruban RH, Kern SE: Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. Am J Pathol 160(1): 91-99, 2002.

Iacobuzio-Donahue CA, Argani P, Hempen PM, Jones J, Kern SE: The desmoplastic response to infiltrating breast carcinoma: Gene expression at the site of primary invasion and implications for comparisons between tumor types. Cancer Res 62(18): 5351-5357, 2002.

Ryu B, Jones J, Hollingsworth MA, Hruban RH, Kern SE: Invasion-specific genes in malignancy: SAGE comparisons of primary and passaged cancers. Cancer Res 61: 1833-1838, 2001.

Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW: Gene expression profiles in normal and cancer cells. Science 276: 1268-1272, 1997.

## Scientists Identify Genetic Variations that may be Associated with Prostate Cancer

*Background*: It would be much easier to know who would benefit from more intensive prostate cancer screening if we knew which genetic mutations were associated with this disease. Several genes have been linked to prostate cancer risk. However individual genes contain a lot of DNA, and researchers haven't yet found the specific mutations therein that allow this cancer to develop. Investigators have looked for these mutations, but due to the genetic complexity of prostate cancer and also to the wide range of research methods used, results have been conflicting.

*Advance*: Conducting careful population and family-based studies, NIH Special Program of Research Excellence (SPORE) investigators found specific variations in several genes that appear to be associated with the development of prostate cancer. These genes are *HSD3B1* and *HSD3B2*, which are important for proper functioning of androgen (a hormone), the *androgen receptor gene* itself, and *hOGG1*, which appears to repair damage to DNA caused by reactive oxygen species (also called free radicals) in the prostate and other body tissues.

*Implications*: These are the first studies to comprehensively study the association of these genetic variations with prostate cancer. Although the data strongly support such an association, the results should be viewed with caution. Further epidemiological and functional studies should be conducted to verify these important findings.

Chang B, Zheng SL, Hawkins GA, Isaacs SD, Wiley KE, Turner A, Carpten JD, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB, Xu J: Polymorphic GGC repeats in the androgen receptor gene are associated with hereditary and sporadic prostate cancer risk. Hum Genet 110: 122-129, 2002.

Chang B, Zheng SL, Hawkins GA, Isaacs SD, Wiley KE, Turner A, Carpten JD, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB, Xu J: Joint Effect of *HSD3B1* and *HSD3B2* Genes Is Associated with Hereditary and Sporadic Prostate Cancer Susceptibility. Cancer Res 62: 1784-1789, 2002.

Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang B, Bleecker ER, Walsh PC, Meyers DA, Isaacs WB: Associations between *hOGG1* Sequence Variants and Prostate Cancer Susceptibility. Cancer Res 62: 2253-2257, 2002.

Chang B, Zheng SL, Isaacs SD, Wiley KE, Carpten JD, Hawkins GA, Bleecker ER, Walsh PC, Trent JM, Meyers DA, Isaacs WB, Xu J. Linkage and association of CYP17 gene in hereditary and sporadic prostate cancer. Int J Cancer 95(6): 354-359, 2001.

## Gene Expression Analysis of Human Chondrocytes

*Background*: Joint cartilage chondrocytes are the highly specialized cells responsible for the production and maintenance of cartilage tissue. This tissue has unique properties allowing it to cushion the ends of the bones and provide a smooth frictionless surface during motion. To produce and maintain a properly functional cartilage, the chondrocyte displays specific patterns of gene expression both during development and in the adult. However, normal chondrocytes show a range of plasticity, as cells isolated from cartilage from different anatomic sites or at different stages of development vary in gene expression. Further alterations in gene expression profiles may occur in injured or diseased chondrocytes, resulting in a functionally impaired tissue. Enhanced understanding of gene expression in chondrocytes and the range of what constitutes normal may lead to the development of strategies to allow the chondrocytes to preserve their function in degenerative joint diseases.

*Advance*: This advance exploits a new technology called microarray gene expression analysis, which is being used in many laboratories to assess gene expression profiles of cells, for analysis of gene expression profiles in human chondrocytes in tissue culture. The results show dramatic differences in gene expression profiles as the chondrocytes are induced to differentiate. These changes encompass a broad range of genes, including those for structural proteins, growth factors, and regulatory factors, as well as many genes not yet reported to be expressed by chondrocytes.

*Implications*: Despite its immense public health impact, treatment of degenerative joint disease is limited to a few classes of medications which provide primarily symptomatic relief and have not been demonstrated to interfere with the progression of disease. The study of gene expression of normal human chondrocytes undergoing differentiation may have important implications for injured and diseased chondrocytes. Such studies expand the science base needed for the development of cell therapies for the correction of joint disease.

Stokes DG, Liu G, Coinbra IB, Piera-Velazquez S, Crowl RM, Jiménez SA. Assessment of the gene expression profile of differentiated and dedifferentiated human fetal chondrocytes by microarray analysis. Arthrit Rheum 46(2): 404-419, 2002.

## Cartilage Injury and Intercellular Signaling for Cell Death: Implications for OA Progression

*Background*: Osteoarthritis (OA) is the most common joint disease and is associated with degeneration of articular cartilage and loss of joint function. It is thought that OA can be caused by mechanical damage to the joint cartilage, and in *in vitro* studies, mechanical damage resulted in OA-like changes in the cartilage. Cyclic mechanical impacts on cartilage explants have been shown to cause physical damage to the tissue matrix and "programmed" cell death, similar to what is seen in OA. Much research has been conducted on what mediates cell death in the chondrocytes during OA, and there is evidence that there are several independent pathways through which cell death can occur. This study was undertaken to further elucidate the mechanisms of cell death in joint injury that may also relate to OA.

*Advance*: Researchers at Cornell University conducted *in vitro* studies on impact loaded cartilage explants. Their studies showed that at an early time following impact loading only cells in the impacted regions underwent cell death, but over time areas of cell death spread to unimpacted regions. However, this effect could be prevented by physical isolation. These findings suggest the existence of intercellular soluble signaling as a cause for cell death in cartilage explant culture.

*Implications*: Considering the correlation between matrix damage and the onset of OA, this study may help us to understand the factors causing OA in cartilage. A loss of chondrocyte viability may play a significant role in the progression of OA, and the possible finding that intercellular signaling is involved in the early onset of OA can provide future researchers with a potential way of intervening in the transmission of the signal, and thus of controlling progression of OA. However, due to the *in vitro* nature of this study, further studies are needed *in vivo* as well as on the nature of the signaling mechanism.

Levin A, Burton-Wurster N, Chen C-T, Lust G: Intercellular signaling as a cause of cell death in cyclically impacted cartilage explants. Osteoarthrit Cartilage 9: 702-711 (2001).

## Differences in Damage Caused by Systemic Lupus Erythematosus among Hispanics, African-Americans, and Caucasians

*Background*: Non-Caucasian populations not only have a higher overall occurrence of systemic lupus erythematosus (SLE), but also seem to have lower survival rates. It is unclear, however, how damage to the various organ systems affected by lupus accrues. To sort out the genetic and socioeconomic/ demographic factors associated with poor outcome in SLE in three major ethnic groups in the U.S., a sample of patients with disease duration of 5 years or less was identified and followed up over time.

*Advance*: Seventy-two Hispanics, 104 African Americans, and 82 Caucasians with SLE were involved in this study for approximately 5 years. Patients in the three ethnic groups had comparable disease duration. At the time of study entry, kidney and cardiovascular damage were more common among non-Caucasians than among Caucasians, and overall measures of disease activity and maximum medication (corticosteroid) dose were higher among non-Caucasians. At the last study visit (at about 5 years of follow-up), about half the patients had accrued some damage. Damage occurred more frequently in Hispanics (61 percent) than in African Americans (51 percent) or Caucasians (44 percent). Kidney damage increased more in Hispanics and African Americans than in Caucasians, and Hispanics had higher overall damage at follow-up than the other groups. Neuropsychiatric problems accounted for the greatest proportion of organ system damage in Hispanics and Caucasians, while hair loss and skin damage accounted for the greatest proportion of damage in African Americans. Overall damage was also higher among patients with: lower socioeconomic status, acute onset of lupus, higher disease activity at diagnosis, higher maximum corticosteroid dose, inadequate coping styles, higher levels of helplessness, and poorer social support.

*Implications*: The finding that about half the patients had not accrued any damage within 5 years of diagnosis probably reflects not only the fact that some patients may have relatively mild disease, but that others who have more severe disease are being treated before damage develops. However, the proportion of patients accruing any damage was higher among Hispanics than among the other two groups, confirming the greater negative impact of SLE among members of this ethnic group. The association of damage with poor coping skills has not been previously reported, and suggests that approaches designed to modify patients' behaviors and attitudes with respect to their illness could lead to reduced damage in SLE. Non-pharmacologic approaches may constitute an essential element in the treatment of patients with SLE if the long-term consequences of this disease and its treatments are to be avoided.

Graciela S. Alarcón, et al.: Systemic lupus erythematosus in three ethnic groups: IX. Differences in damage accrual. Arthritis & Rheumatism 44(12): 2797-2806, 2001.

## Insulin Resistance in Myotonic Dystrophy

*Background*: Myotonic dystrophy (DM) is the most common form of muscular dystrophy associated with adult onset. The disease is characterized by multiple tissue involvement, including muscle hyperexcitability, progressive muscle wasting, and alterations in smooth muscle function. Insulin resistance (the inability to use insulin) is a common metabolic abnormality in type 1 myotonic dystrophy (DM1). Patients with DM1 do not respond to insulin infusion as well as normal subjects, but do not have the clinical symptoms of type 2 diabetes.

The mechanism of insulin resistance in DM1 is unknown, though evidence suggests that the cause is alteration in receptor function (that is, the cell does not recognize insulin and thus cannot use it). There are two predominant forms of insulin receptor. One form, insulin receptor B (IR-B) is expressed predominantly in insulin-responsive tissues that are responsible for regulating blood sugar levels, namely skeletal muscle, liver, and adipose tissue. Insulin receptor A (IR-A) is expressed in other tissues.

*Advance*: Researchers have found that muscles in DM1 patients have inappropriately high levels of the non-muscle insulin receptor, IR-A. This is apparently due to abnormal regulation of cell production of the insulin receptor in DM1 patients. The misregulation is most likely due to unusual genetic messages associated with the genetic defect. This conclusion is confirmed by studies with mice muscle cells in culture. When the researchers added high levels of the erroneous genetic messages, the muscle cell cultures began producing non-muscle insulin receptor A. This modification of the cells resulted in lower levels of insulin stimulated sugar uptake, even though there was no loss in the number of insulin receptors.

*Implications*: This finding suggests a direct mechanistic link between the gene defect in myotonic dystrophy and one of the wide array of symptoms associated with the disease. Continued research on the subject should provide better understanding of the diffuse nature of the disease and suggest ways to treat the different symptoms.

Savkur RS, Philips AV, Cooper TA: "Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy." Nat Genet 29(1): 40-47, 2001.

## Abnormal Accumulation of Gene Messages in Myotonic Dystrophy

*Background*: Myotonic dystrophy (DM) is the most common form of congenital muscular dystrophy associated with adult onset. The disease is characterized by multiple tissue involvement, including muscle hyperexcitability (a condition which can result in muscle spasms at the onset of sudden movement) and progressive muscle wasting. In most families, the disease is associated with how many times a specific series of amino acids (known as the triplet nucleotide, or CTG) is repeated on Chromosome 19. Disease severity increases as the size of the expansion (i.e., the number of repeats) increases. The expansion occurs in a region of the genetic material that usually is considered to be non-functional. This form of the disease is called myotonic dystrophy 1 (DM1). Recently a second form of the disease, myotonic dystrophy 2 (DM2), has been shown to be due to a defect on chromosome 3. DM2 appears to be caused by an expanded "CCTG" repeat, rather than the CTG repeat in DM1. Manifestations of both forms of the disease are similar, though initial weakness is usually in different muscles. One hypothesis for the pathogenesis of DM is that copies of the expanded region interfere with normal expression of unrelated proteins.

*Advance*: Researchers using fluorescence staining found that there are abnormal clumps (foci) of genetic material in the nuclei of cells from DM patients. The staining was based on the triple repeat found in DM1, indicating that the accumulated material consists of copies of the expanded region. The clumps were found in all DM1 and DM2 patients exhibiting symptoms. It is likely that nuclear retention of this protein plays a role in DM pathogenesis. Since the protein is retained in the nucleus in both DM1 And DM2, it suggests a common pathway even though the gene defects associated for the two forms of the disease are on separate chromosomes.

*Implications*: The results suggest that a considerable portion of the symptoms in DM are associated with the accumulation of genetic messengers within the nuclei of muscle cells. This finding shows that these foci bind a protein that may be needed for normal muscle development. More work is needed to find the exact role of this protein in humans and to find if other proteins similarly accumulate in cell nuclei rather than move to their normal functional location within cells. This knowledge would help considerably in treating DM.

Mankodi A, Urbinati CR, Yuan QP, Moxley RT, Sansone V, Krym M, Henderson D, Schalling M, Swanson MS, Thornton CA. "Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2." Hum Mol Genet 10(19): 2165-2170, 2001.

## The Scientific Basis for the Use of Electrical Current to Heal Fractures

*Background*: Electrical currents as stimulation have been used to heal fractures that have failed to heal for almost 200 years. More recently, electrical stimulation modalities have been used to augment spinal fusions (a surgical procedure to allow one spinal bone to unite with an adjacent one). Despite this usage, we do not fully understand the biology and physics behind these uses. A recently reported study provides further insights into these unknowns.

*Advance*: Using cultured bone cells, researchers assessed the biological response of bone cells to three types of electrical stimulation in common clinical use today (capacity coupling, inductive coupling or combined electromagnetic fields). The biological response studied was an increase in DNA content as a marker for increased cell activity. Compared to controls that did not receive any electrical stimulation, all three types of electrical stimulation increased bone cell activity. In addition, the response of the bone cells significantly increased when exposed to capacity coupling stimulation. In addition, the effects seen occurred at different locations within the cell and by different biochemical/molecular pathways. The final pathway or step for all three is the same. Finally, the differences seen in bone cell proliferation are due to differences in signal communication within the cultured bone cells.

*Implications*: This study provides valuable insight into the biology and physics behind the use of electrical stimulation to heal fracture nonunions and augment spinal fusions. Further scientific developments may result in the optimization of this technology for use in fracture treatment and fusion surgery.

Brighton, CT, .: Signal Transduction in Electrically Stimulated Cells . Journal of Bone and Joint Surgery, 83-A (10): 1514-1523, 2001.

## Understanding the Molecular Basis of the Hair Cycle and Hair Loss

*Background*: Hair is a complicated cycling structure in skin. There are a number of hair loss diseases, most of them acquired but some hereditary. Understanding the molecular basis of normal hair growth and cycling is greatly aided by investigating the rare genetic hair loss diseases and also by the use of animal model systems.

*Advance*: One of the genetic diseases of hair loss is termed Atrichia. This is a disease in which the hair is lost shortly after birth and does not redevelop. Cysts also develop in the hair bearing areas. This disease was previously shown to be the human equivalent of a mouse model system widely used in studying skin diseases and a specific gene, termed the hairless gene, was shown to be the basis of both the human and mouse diseases. In the current study, a group of investigators determined that an identical disease was seen in an individual who had a different genetic defect. In this case, rather than the hairless gene being defective, the defective gene was the gene for the vitamin D receptor. Both of these genes code for proteins that have similar structures and it appears that they both may participate in the same pathway controlling the cycling of hair after birth.

In another study also looking at hair cycling, a group of investigators looked at the molecules involved in hair cycling after birth. They compared this with the initial development of hair in embryonic skin. The same molecules are active in both the initial embryonic induction of the hair follicle and in the cycling of the new growing phase hair follicle after birth. Specific molecules were identified that both initiate the growth and can inhibit the growth of the hair follicles and implicate a particular pathway as being important and/or essential in both the embryonic initiation of the hair follicle and in the cycling of hair after birth.

*Implications*: These studies of the molecules involved in hair growth and cycling and in diseases of hair improve our understanding of this complicated mechanism. They have the potential to provide treatments for hair diseases including the hair loss side effects that often accompany chemotherapy for various malignancies.

Miller J, Djabali K, Chen T, Liu Y, Ioffreda M, Lyle S, Christiano AM, Holick, M, Cotsarelis G: Atrichia caused by mutations in the vitamin D receptor gene is a phenocopy of generalized atrichia caused by mutations in the hairless gene. JID 117(3): 612-618, 2001.

Botchkarev VA, Botchkareva NV, Nakamura M, Huber O, Funa K, Lauster R, Paus R, Gilchrest: Noggin is required for induction of the hair follicle growth phase in postnatal skin. FASEB: 2205-2214, 2001.

## Function of the Ichthyosis-Related Molecule Filaggrin in the Skin

*Background*: Filaggrin is a molecule that has been identified as functioning in the development of the outermost layer of skin. It has been implicated as being important in the abnormalities seen in the group of diseases termed ichthyosis, in which there is excessive scaling and abnormal function of this outer layer of skin. The normal function of filaggrin is to associate other molecules normally found in skin together to form the major structural component of the outermost layer of skin. However, it is becoming clear that, as with other molecules in human diseases, filaggrin has more than one function.

*Advance*: In a recent study, a group of investigators examined the function of filaggrin as it interacts with the structural elements within the cells of the epidermis. These structural elements form the internal "skeleton" of the cell, often called the cytoskeleton, and include several molecules, keratin filaments, actin, microfilaments and microtubules. These are all molecules involved in giving the cell a three dimensional structure and allowing it to progress through its normal layered maturation to the eventual formation of the outermost dead layer of skin. Using a model system in which filaggrin could be selectively turned on, it was demonstrated that excess filaggrin produced at the wrong time dramatically changed the cellular architecture and interfered with the normal maturation of the cells. These studies demonstrate that filaggrin has effects on several different molecules involved in cellular structure and function.

*Implications*: Understanding the molecular events in the normal maturation of skin and in the normal and diseased development of the outermost layer of skin is important not only in developing treatments for ichthyosis but also for developing methodologies to allow therapeutic molecules to pass through the outer layer of skin and be more readily absorbed. As filaggrin plays a major role in normal and abnormal development of the barrier of skin, a better knowledge of exactly how this role is played will not only help our understanding of certain skin diseases but may provide an avenue for the development of new delivery vehicles for topically applied medications.

Presland RB, Kuechle MK, Lewis SP, Fleckman P, Dale BA: Regulated expression of human filaggrin in keratinocytes results in cytoskeletal disruption, loss of cell-cell adhesion and cell cycle arrest. Exp Cell Res 270(2): 199-213, 2001.

## Understanding the Molecular Basis for Lethal Versus Non Lethal Types of Epidermolysis Bullosa

*Background*: The so-called "junctional" forms of the severe hereditary blistering disease epidermolysis bullosa are associated with abnormalities in a particular molecule termed laminin 5. Some forms of this disease are typically fatal during the first year of life, whereas other forms of the disease are much milder and allow the children to live a much more normal life. Understanding why certain changes in laminin 5 are inconsistent with life and others allow the baby to survive has been the focus of a good deal of research over the last few years.

*Advance*: A group of investigators looked at twenty-seven families where the disease junctional epidermolysis bullosa was present. Fifteen of the families had the Herlitz or typically rapidly fatal form of the disease and twelve showed the less severe non-Herlitz variety. The particular mutations in the molecules that make up laminin 5 were investigated. There were different types of mutations found in the fatal, or Herlitz form of the disease, as compared to the non-fatal form. Typically in the Herlitz disease, the protein laminin 5 was not produced because a mutation called a premature termination codon. In the non-fatal forms of the disease, there were other mutations but these mutations did allow some protein to be produced and, therefore, the disease was less severe.

*Implications*: The tools of the molecular genetics are rapidly advancing. We are developing the ability to identify the genes associated with disease and, specifically, identify the mutations in these genes. However, until we also know or can predict whether the mutations will result in severe or mild disease, simply identifying the fact that a mutation exists is much less useful for family planning or a better understanding of the disease process. Studies of this variety, in which large groups of individuals with different severity of the same disease and with known mutations can be analyzed, will help provide that information.

Nakano A, Chao S-C, Pulkkinen L, Murrell D, Bruckner-Tuderman L, Pfendner E, Uitto J: Laminin 5 mutations in junctional epidermolysis bullosa: molecular basis of Herlitz vs non-Herlitz phenotypes. Hum Genet110(1): 41-45, 2002.

## Understanding Desmosome Structure and Function

*Background*: Desmosomes are structures in the epidermis that function as major attachment sites allowing skin cells to attach to one another. There are a relatively large number of molecules that go into making up the desmosome and there are variations in particular molecules in desmosomes in different parts of the body. These desmosomes or their component proteins are the target of autoimmune attack in the pemphigus group of diseases. Understanding the structure and function of desmosomes is important to understanding the diseases involved and designing approaches to ameliorate the effects of the disease.

*Advance*: One of the molecules making up desmosomes is called desmoglein 3. This molecule is attacked by antibodies in the severe blistering disease pemphigus. In this study, the function of desmoglein 3 was investigated using models in which different parts of the molecule had been modified. It was determined that the part of the molecule that associates with another desmosomal molecule called plakoglobin is important in helping to organize the desmosome. In the absence of this association, the molecule is not incorporated into the desmosome properly and the desmosome does not function properly.

Another molecule found in the desmosomes is termed desmoplakin. A group of investigators used a mouse model in which this molecule was eliminated to investigate its function. With the desmoplakin eliminated, the desmosomes in these cells looked normal but were unable to interact with one of the cellular structures termed keratins. With the inability to interact with this cellular component, the epidermal cells were unable to form normal structures called epidermal sheets under the experimental conditions. This indicates that this molecule and these interactions are essential for the normal function of the desmosome.

*Implications*: Understanding the structure and function of the epidermis and its components is essential in understanding how skin as a whole functions and how the skin is abnormal in certain diseases. It will also lead to new potential interventions in the treatment of these diseases.

Andl CD, Stanley JR: Central role of the plakoglobin-binding domain for desmoglein 3 incorporation into desmosomes. JID 117(5): 1068, 2001.

Vasioukhin V, Bowers E, Bauer C, Degenstein L, Fuchs E: Desmoplakin is essential in epidermal sheet formation. Nat Cell Bio 12: 1076-1085, 2001.

## Understanding the Basic Biology of Keloids

*Background*: Keloids are an abnormal form of scarring at areas of injury. They are much more common in African Americans and can be quite large and disfiguring. Keloids are distinguished from normal scars in that keloids continue to grow beyond the border of the original injury and for an extended period of time. No entirely satisfactory method of treatment exists.

*Advance*: Based on a clinical observation that a keloid cleared up in a patient under study for the use of the topical drug tacrolimus for atopic dermatitis, a study was undertaken to look at the potential basis for this effect. In this study a protein termed Gli-1 (which is the product of a tumor associated gene) was investigated. It was determined that the protein was found in excess amount and that the gene was more active than in normal controls.

*Implications*: The excess presence of the protein and the overactivity of the gene raise the potential that this is an important component to the development of keloids. Interventions that inhibit the gene or the protein may provide the potential for a new avenue of treatment. Given the lack of good treatments for keloids and the relatively simple and nonscarring nature of topical tacrolimus treatment, this would be a major therapeutic advance if substantiated in additional studies.

Kim A, DiCarlo J, Cohen C, McCall C, Johnson D, McAlpine B, Quinn AG, McLaughlin ER, Arbiser JL: Are keloids really gli-loids?:high-level expression of gli-1 oncogene in keloids. J Am Acad Dermatol45(5): 707-711, 2001.

## The Use of Large Scale Gene Expression Studies in Investigating Psoriasis

*Background*: Psoriasis is a common skin disease affecting approximately 2 percent of the population. It is clear that it is primarily a disease in which the immune system drives the skin to produce an excessively thickened and abnormal skin recognizable clinically as psoriasis. There are probably environmental risk or trigger factors as well. Studies of populations have narrowed the area in which the psoriasis susceptibility gene is located to certain portions of certain chromosomes but, despite many years of study, specific genes have not yet been identified.

*Advance*: New technologies now allow for the simultaneous investigation of the activity of thousands of genes and/or gene products at a single time. Among the limitations of these approaches are the huge amounts of information that are generated and, also, the potential that despite the many molecules available for testing, the molecules of greatest interest may not be present in the commercially available systems. Using a system that examines 12,000 genes at a time, the involved and uninvolved skin of 15 psoriatic patients and 6 normal individuals was studied. A number of differences were observed both between the psoriatic individuals and the normals and between the uninvolved and involved skin of the psoriasis patients. A total of 177 differences were found between the psoriatic and normal individuals, 10 of which were also found to be different between the involved and uninvolved skin of the psoriasis patients. Several of these mapped to regions of the human genome that had previously been identified as psoriasis susceptibility sites.

*Implications*: These studies provide evidence that the developing technology of genomic and proteomic screening can be used to investigate psoriasis and, potentially, other skin diseases as well. The results from this line of research do seem to be consistent with that from other lines of research and may provide a quicker easier means for identifying the specific genes involved rather than the more laborious approach of fine mapping of the previously identified psoriasis susceptibility areas of the chromosomes.

Bowcock AM, Shannon W, Du F, Duncan J, Cao K, Aftergut K, Catier J, Fernandez-Vina MA, Menter A: Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. Hum Mol Genet 10(17): 1793-1805, 2001.

## Molecular Basis and Animal Model System of Atopic Dermatitis

*Background*: Atopic dermatitis is a common, chronic and inflammatory skin disease that occurs more often in the pediatric population than in adults. Its prevalence has been increasing, particularly in industrialized societies over the last fifty years. In some studies it has a prevalence of up to 20 percent of children in urban settings. Research has been hampered by a lack of a good animal model for atopic dermatitis. It is known to be an immunologically mediated disease with abnormalities in various of the mediators of the inflammatory response but it is not clear whether any of these changes are the cause of the disease or simply a reflection of ongoing disease. The presence of atopic dermatitis in the individual or their family contacts is a contraindication to smallpox vaccination, a major bioterrorism consideration.

*Advance*: One team of investigators generated a transgenic mouse in which an inflammatory mediator termed interlukin-4 was produced at increased concentrations. These mice developed an inflammatory itchy skin disease that duplicated all the key features of human atopic dermatitis. The disease developed early in life and many of the factors seen in the blood in human patients were also seen in these animals as well. The mice developed the disease at a 43 percent incidence which, while greater than that seen in the human population, is in the same general range. Other researchers investigated the white blood cells and skin lesions of patients with atopic dermatitis. In this study, they focused on the molecule interlukin-15, a molecule thought to play a major role in atopic dermatitis. They demonstrated that interlukin-15 is reduced in atopic patients as compared to other disease controls and that this reduction in interlukin-15 may be a component of the development of the manifestations of atopic dermatitis.

*Implications*: These studies will help us understand the abnormalities resulting in atopic dermatitis. The availability of an animal model will allow more detailed studies as well as provide a system in which therapeutic interventions can be tested. This has great potential for advancing our ability to treat this common often severe skin disease. It will also help to develop an approach to smallpox vaccination in atopic families, if that were to become a public health necessity.

Chan LS, Robinson N, Xu L: Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol117(4): 977-983, 2001.

Ong, PY, Qutayba AH, Travers JB, Strickland I, Muhamed AK, Boguniewicz M, Leung DYM: Decreased IL-15 may contribute to elevated IgE and acute inflammation in atopic dermatitis. J Immuol168(1): 505-510, 2002.

## Children with Juvenile Rheumatoid Arthritis Do Not Have Permanent Knee Damage Early in the Disease

*Background*: Juvenile rheumatoid arthritis (JRA) is the most common rheumatic disorder of childhood, and the knee is the most frequently affected joint. Conventional x-rays are of limited use early in the disease because they do not show the inflamed tissue, cartilage destruction, and early erosion of the bone. Magnetic Resonance Imaging (MRI) allows these changes to be observed.

*Advance*: MRI was conducted on knees of children who had JRA for a year or less in order to see the earliest changes in the affected joint. Ninety-seven percent of the affected knees showed swelling of the knee and commonly the membrane of the knee joint was thickened. Although damage to the cartilage and bone of the knee were occasionally seen, this was not a common finding. Destructive changes in cartilage and bone were seen only in patients with mature skeletons. This finding, combined with the observation that joint cartilage thickness decreases with age, supports the speculation that thicker cartilage, along with intact growth cartilage blood supply and better repair processes in skeletally immature children, may account for the fewer erosive changes seen in JRA compared with those seen in adult rheumatoid arthritis.

*Implications*: This study suggests that children with JRA are less likely to suffer joint damage early in the disease than adults. With more effective drugs to treat rheumatoid arthritis now available, this suggests that early aggressive treatment may prevent or ameliorate development of permanent joint damage in JRA.

Gylys-Morin, VM, Graham TB, Blebea JS, Dardzinski BJ, Laor T, Johnson ND, Oestreich AE, Passo MH: Knee in Early Juvenile Rheumatoid Arthritis: MR Imaging Findings. Radiology 229: 696-706, 2001.

## Molecular Basis for Differences between Human Joints

*Background*: Osteoarthritis (OA) is a slowly developing joint disease that affects at least 50 million adults in the United States alone and approximately 15 percent of the world's adult population with pain and disability. For as yet poorly understood reasons, the prevalence of OA is consistently higher in some joints than in others. The majority of patients are symptomatic in one joint only, with certain joints being affected more frequently than others. In the lower extremity, the hips, the knees, and the joint of the big toe are most commonly affected. Other joints, such as the ankle are spared.

*Advance*: Investigators have studied the metabolic and biochemical basis of the differences between the chondrocytes of the knee and ankle. Chondrocytes are the cells in the cartilage that surround joints responsible for maintaining the molecular matrix of cartilage. Cartilage matrix is chiefly composed of proteins called collagens and proteoglycans.

The investigators harvested human cartilage from the knee and ankle for study through the Regional Organ Bank of Illinois. They found that there are differences between the matrix components and water content of cartilage in knee and ankle. These differences correspond to a greater stiffness and lower permeability in the ankle to make the ankle cartilage stiffer and slowing movement of molecules through the cartilage. The chondrocytes of the knee respond more strongly to stimulus by depressing the synthesis of matrix and increasing enzymatic activity degrading matrix. These responses inhibit cartilage repair. In addition, the bone underlying the cartilage becomes much denser with damage in the knee. This denser bone may also contribute to cartilage degradation, although how this relationship works is not clear. No such changes in bone density are seen in the ankle with cartilage degradation.

*Implications*: The results of this research have provided evidence that the lower frequency of OA in the human ankle than in the knee may be due to a combination of factors including metabolic, biochemical, and biomechanical differences between the cartilages from the two joints. By expanding our understanding of how the two cartilages differ from one another, we may be able to identify early stages of damage that may precede OA. The goal in OA research is to identify the early stages of disease, develop means of blocking the progress of the disease process, and reversing its effects.

Cole AA, Kuettner KE: Molecular basis for differences between human joints. Cell Mol Life Sci 58:1-8, 2001.

Muehleman C, Berzins A, Koepp H, Eger W, Cole AA, Kuettner KE, Sumner DR: Bone density of the human talus does not increase wit the cartilage degeneration score. Anat Rec 255(2): 81-86, 2002.

## Heart Disease and Osteoporosis May Be Related Through a Common Mechanism

*Background*: Atherosclerosis, caused by calcification of major vessels, and osteoporosis are major sources of morbidity and mortality in both men and women. Recent evidence from animal models have added an exciting new dimension to the study of vascular calcification and bone cell regulation. Osteoprotegerin (OPG), a key protein in bone cell regulation, may also play a role in extra-skeletal calcification. Mice missing osteoprotegerin protein through genetic manipulation are osteoporotic and also exhibit premature aortic calcification. Other key bone proteins, osteopontin and matrix Gla protein, are also expressed in vascular tissue and appear to be key factors in inhibiting the mineralization of vascular tissue. In mice engineered to lack matrix Gla protein, accelerated vascular calcification kills the mice within weeks.

*Advance*: The Framingham Heart Study is a population-based longitudinal cohort of older men and women. In conjunction with the companion NIH-supported Framingham Osteoporosis Study this cohort offers an unparalleled mechanism for scrutinizing the incidence of cardiovascular disease and osteoporosis over many years, as well as the opportunity to explore any relationship between the two diseases.

Vascular calcification can be detected and displayed on routine lateral lumbar spine radiographs as dense calcium mineral deposits of the aorta that lie adjacent to osteopenic (low bone density) vertebrae. Bone loss and osteoporosis can be determined from hand x-rays. Investigators explored the hypothesis that the progression of vascular calcification of the abdominal aorta should be greatest in those individuals with the greatest amount of bone loss. Both calcification and bone loss were assessed in radiographs and hand x-rays taken approximately 25 years apart. There was a significant association between bone loss and change in aortic calcification index in women after controlling for all potential confounders. No association was observed in men, including the 50 percent of men with the greatest bone loss. Men lost less bone in general and the power to detect a difference may have been limited in men.

This is the first longitudinal study to show that women with the greatest magnitude of bone loss also demonstrate the most severe progression of abdominal aortic calcification, suggesting that the two processes may be related.

*Implications*: The exciting possibility that these two diseases are related suggests that a common mechanism can be manipulated for enhanced therapeutic effect in the treatment of both conditions. The fact that a relationship between bone loss and vascular calcification could be demonstrated in women but not men is puzzling but could suggest important gender differences in the pathophysiology of the disease processes.

Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW: Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. Calcif Tissue Int 68(5): 271-276, 2001.

## Rheumatoid Arthritis Patients at Increased Risk for Cardiovascular Events

*Background*: Patients with rheumatoid arthritis (RA) have a shortened lifespan. As in the general population, the most frequent cause of death in RA is cardiovascular (CV) disease. However, CV mortality in persons with RA occurs in excess of what would be expected in people without RA. Disease severity has been associated with CV mortality in RA, but it is not clear if this association is attributable to a higher-than-normal frequency of CV events or a higher-than-normal case fatality rate when CV events occur. An increased prevalence of atherosclerosis in RA may be suspected for several reasons: atherogenic side effects of some antirheumatic medications, the effects of chronic systemic inflammation on the vascular endothelium, or shared mechanisms between RA and atherosclerosis. Recent evidence suggests that systemic inflammation may play a role in the development of atherosclerosis. It is also possible that RA and atherosclerosis share pathogenic mechanisms. In view of this evidence, accelerated atherosclerosis in RA should be expected.

*Advance*: Investigators found that CV events occurred more frequently than would be expected in RA, and that this increased incidence is not entirely explained by traditional CV risk factors. The number of CV events observed in RA patients was almost 4 times higher than what would be expected in persons without RA of the same age and sex. The CV risk factor profile of RA patients has not been studied thoroughly. Previous studies of CV disease in RA have focused on fatal CV events. This is one of the first studies of CV risk in RA that focuses on both fatal and nonfatal events.

*Implications*: Physicians who provide medical care to patients with RA should recognize that this disease is characterized by a predisposition to atherosclerosis and CV disease and should implement appropriate prophylactic or therapeutic measures, as clinically indicated.

Del Rincón I, Williams K, Stern M, Freeman G, Escalante A: High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum44(12): 2737-2745, 2001.

## Pattern of Inheritance of Familial Keloids Identified

*Background*: Keloids are a wound-healing disorder. They are described as proliferative fibrous growths that result from an excessive tissue response to skin trauma. Most keloids occur sporadically, but some cases are familial. However, the genetics of keloid formation have only rarely been documented, and the mode of inheritance is not known. The epidemiology of keloids in general is variable. The reported incidence of keloids in the general population ranges from a high of 16 percent among the adults in Zaire to a low of 0.09 percent in England. It is widely accepted that darker-skinned populations have a higher incidence of keloid formation than lighter-skinned populations.

*Advance*: The pattern of inheritance (technically termed to be consistent with an autosomal dominant mode with incomplete clinical penetrance and variable expression) of familial keloids has been reported for the first time. These findings were based on the clinical and genetic characteristics of 14 families that were studied across at least three generations. Some of the family members had only minor earlobe keloids, whereas others had very severe keloids affecting large areas of the body.

In the study reported here, most of the families were of African American ethnicity, supporting a higher incidence of keloid formation in darker-skinned rather than in lighter-skinned populations. This might, however, be a result of different ethnicity rather than different skin color, since some of the lighter-skinned members of these African American families developed more severe keloids than their darker-skinned relatives. The varying data might be explained in part by the many factors influencing keloid formation, such as ethnicity, age, anatomic location, and type of trauma.

*Implications*: This is the most comprehensive collection of keloid families described to date, and it allows for the first time the understanding of the clinical and genetic characteristics of the familial form of this wound-healing disorder.

Marneros A, Norris J, Olsen B, Reichenberger E: Clinical Genetics of Familial Keloids. Arch Dermo137(11): 1429-1434, 2001.

## New Insights Regarding Cardiovascular Disease and Osteoporosis in Women with Systemic Lupus Erythematosus

*Background*: Systemic lupus erythematosus (SLE) is a systemic, inflammatory autoimmune disease that affects predominantly young, premenopausal women. Because survival has improved over the last 2 decades, investigators are paying increasing attention to complications leading to late mortality and progressive morbidity. Increasing numbers of young women with SLE are experiencing fractures, strokes, and heart attacks, complications typically associated with aging in non-SLE populations of women. The risk of fracture was increased 5-fold in a large cohort of lupus patients whose mean age was less than 45 years. Similarly, the risk of myocardial infarction reached 50 times higher than expected in women with SLE who were ages 35-44 years, a population that should otherwise be protected from such risks.

Experimental evidence suggests that inflammation and immune-mediated mechanisms, key factors in the pathogenesis of lupus, play a role in bone formation and heart disease. This study investigated whether bone mineral density (BMD) was associated with the carotid plaque index, or coronary artery calcium score in lupus patients.

*Advance*: Investigators demonstrated an association between decreased bone mineral density and both an increased carotid plaque index and presence of coronary artery calcification in a cohort of young women with lupus. Evidence from this study supports a role for traditional risk factors, such as sedentary lifestyle, hypertension, and hyperlipidemia, in the premature development of osteoporosis and atherosclerosis in patients with SLE. Corticosteroids, commonly used in the treatment of SLE, likely contribute to these morbidities. Investigators hypothesize that the underlying link to osteoporosis and cardiovascular disease is also related to the inflammatory and immunologic nature of SLE.

*Implications*: These findings, that women with SLE are at increased risk for both clinical osteoporotic and cardiovascular outcomes at a much younger age, suggest that this group of women may provide an ideal population in which to study the underlying relationship between these conditions independent of age.

Ramsey-Goldman R, Manzi S: Association of osteoporosis and cardiovascular disease in women with systemic lupus erythematosus. Arthritis Rheumatism 44(10): 2338-2341, 2001.

**Differences Found in Clinical Manifestations of Systemic Lupus Erythematosus in Caucasians in Rochester, Minnesota, when Compared to Chinese in Singapore**

*Background*: Systemic lupus erythematosus (SLE) is a multisystem disease of unknown etiology and worldwide distribution. In the United States, an increased age-adjusted mortality from SLE has been observed among Chinese and other Asian SLE patients compared with white SLE patients. Comparing disease manifestations in Caucasian and Chinese SLE patients could provide insights into reasons for this observed difference in mortality. Investigators hypothesized that the prevalence of proteinuria (protein in the urine) or of central nervous system (CNS) or other major organ involvement at diagnosis of SLE might be higher among Chinese than among Caucasian SLE patients, and that the time to development of such organ involvement might be shorter in Chinese than in Caucasian SLE patients. They studied these hypotheses by comparing the clinical manifestations in 2 already assembled cohorts of SLE patients in Rochester, Minnesota, and in Singapore.

Ethnic differences in SLE clinical manifestations have been observed in studies of several ethnic groups, though the contribution of ethnicity per se to these differences is controversial. For example, African Americans have been found to have increased renal involvement in some but not in other studies. In the most comprehensive study of factors influencing manifestations of SLE to date, the LUMINA (Lupus in Minority Populations, Nature Versus Nurture) investigators have found that genetic and ethnic factors influenced SLE manifestations at diagnosis and that genetic, socioeconomic, and disease-related factors influence development of SLE-related damage in a cohort of Hispanic, African American, and Caucasian SLE patients.

*Advance*: Investigators found that Chinese SLE patients showed a trend toward a higher risk of developing proteinuria or CNS or other major organ involvement over the course of the disease than did Caucasian SLE patients. This trend persisted after adjusting for demographic, socioeconomic, disease-related, and therapy-related variables and may contribute to the increase in mortality seen in Chinese SLE patients. These data may also suggest ethnic differences in SLE disease progression.

*Implications*: The study presented may lead to more appropriate treatment of Asian lupus patients in the US. More broadly, epidemiologic studies such as this one comparing ethnic groups may generate hypotheses related to a greater understanding of SLE etiology.

Thumboo J, Uramoto K, O'Fallon WM, Fong KY, Boey ML, Feng PH, Thio ST, Gabriel SE, Chng HH, Howe HS, Koh ET, Koh WH, Leong KH, Leong KP: A comparative study of the clinical manifestations of systemic lupus erythematosus in Caucasians in Rochester, Minnesota, and Chinese in Singapore, from 1980 to 1992. Arthritis Rheum 45(6): 494-500, 2001.

## Progressive Muscle Weakening Caused by Damaged DNA Synthesis Enzyme

*Background:* Progressive external ophthalmoplegia (REO) is a degenerative disease in which eye muscles deteriorate and the patient must move his head, rather his eyes, to follow an object. It is an inherited disorder and, although a rare affliction, can bring insight into similar diseases that act by affecting mitochondria. Mitochondria are the main energy-producing components of cells and are unique in having their own genes that is distinct from the genes of the rest of our cells. They act semi-independently from the rest of the cell, and they contain a set of 37 specialized genes on a tiny chromosome. Mutations in any of the mitochondrial genes can cause a cellular energy deficit, the first symptoms of which is often the muscular degeneration known as progressive external ophthalmoplegia. Recently, this disease was linked to a mutation (called Y955C) in one of the cell's genes that produces the enzyme (DNA polymerase gamma) that is responsible for replicating mitochondrial DNA.

*Advance:* This study showed for the first time that a damaged enzyme – a DNA synthesis enzyme – played a role in a degenerative disease and presented evidence of how and why an inherited, degenerative disease generally gets worse with time. In this paper, a genetic mutation coding for a conserved tyrosine in the nuclear gene for the mitochondrial DNA polymerase, DNA polymerase gamma, was shown to be associated with the disease. This specific mutation decreased the fidelity of DNA replication and accelerated the accumulation of point mutations, frameshifts, and deletions in mitochondrial DNA. The steady accumulation of mutations is what accounts for the late onset and progressive nature of PEO.

*Implications:* From this work, we now know that an errant mitochondrial DNA polymerase can cause human disease. Understanding that in the replication of a subset of genes (the mitochondrial genes) a damaged enzyme makes errors ten-fold to 100-fold more frequently than in healthy individuals can potentially lead us to ways to prevent the accumulation of the mistakes causes the muscle weakness to progress.

Ponamarev MV, Longley MJ, Nguyen D, Kunkel TA, Copeland WC: Active site mutation in DNA polymerase associated with progressive external ophthalmoplegia causes error-prone DNA synthesis. J Biol Chem 277(18): 15225-15228, 2002.

## Hormonal and Reproductive Risk Factors for Systemic Lupus Erythematosus

*Background:* NIH scientists have been studying potential causes of the autoimmune disease systemic lupus erythematosus (SLE). SLE is a chronic inflammatory autoimmune disease characterized by the production of non-organ-specific autoantibodies. Significant health consequences include renal failure, vasculitis, thrombosis, and seizures and other neurologic complications. SLE disproportionately affects women and African Americans. The incidence and prevalence of SLE are at least three times higher in African Americans and African Caribbeans compared with whites; at least 85 percent of patients with SLE are women. Experimental studies in mouse models of SLE demonstrated exacerbation of the disease by estrogen and prolactin and amelioration by androgens, but few epidemiological studies have examined hormonal or reproductive risk factors for SLE in humans.

*Advance:* The Carolina Lupus Study is a population-based cohort in eastern and central North Carolina. The study examined pregnancy history, use of hormones, and markers of endogenous sources of exposure to estrogen and prolactin (e.g., age at menarche, age at natural menopause, history of breast-feeding). In this study, little or no evidence was found that estrogen-related exposures (e.g., hormone replacement therapy or oral contraceptive use) could be associated with SLE. In fact, SLE was associated in this study with earlier age at natural menopause, the opposite of what would be expected if SLE risk were associated with higher estrogen levels. Age at menarche was not related to SLE risk. Breast-feeding was associated with a decreased risk of SLE.

*Implications:* This study contradicts other reports of an association between long-term use of hormone replacement therapy and development of SLE (which may have been partly confounded by the failure of the earlier study to include age at natural menopause in the analysis.)

Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS: Hormonal and reproductive risk factors for development of systemic lupus erythematosus. Arthritis Rheum 46(7): 1830-1839, 2002.

## Anthrax Invades and Evades the Immune System to Cause Widespread Infection

*Background:* The popular press has been filled with reports of anthrax exposure since September 11th. Usually a disease that strikes mostly livestock and wild animals, it has become a household word since its use as a weapon of terror. The most severe form of the disease results from inhalation of *Bacillus anthracis* spores which are engulfed or phagocytised by macrophages in the lung. Phagocytosis of bacteria by macrophages is a normal and effective method of the innate immune system to fight the spread of infection. However, in the case of anthrax, the bacteria survive phagocytosis, reproduce within the cells, and use the macrophages as a transport mechanism to invade lymph nodes and eventually the blood stream leading to widespread infection, disease, and death. Until now, the mechanisms by which *B. anthracis* kills macrophages and avoids detection by the host immune system has been unclear.

*Advance:* NIH-supported researchers at the University of California at San Diego have discovered that *B. anthracis* evades the host immune system, using a toxin called lethal factor (LF) to destroy macrophages and spread throughout the body. Apparently LF cleaves a mitogen activated kinase (MAPK) kinase that activates p38 MAPK by a cellular process known as phosphorylation. If p38 is not activated inside the macrophage the cell dies by apoptosis rather than proliferatng. This is quite common in the immune system; if a cell doesn't get all the right signals for proliferation it dies. Since the macrophages do not proliferate, their ability to secrete the signaling agents that rev up the immune system is greatly reduced and thus the natural immune system does not mount the necessary defense to fight the infection. The investigators speculate that many other pathogenic bacteria probably use this approach.

*Implications:* These results may explain why anthrax infections proceed nearly undetected until the patient is very sick and near death. Since the cascade of events leading to infection is now clearer, this research may clear a path to the discovery of a drug or agent to block the action of LF and therefore, give the immune system, and other therapeutic agents, more time to detect the infection and fight it. Future research by these investigators will focus on the intricate balance of macrophage activation and death since it seems to play a key role in the ability of the bacteria to spread, multiply, and set up a deadly systemic infection.

Jin Mo Park, Florian R, Greten, Zhi-Wei Li, Michael Karin: Macrophage Apoptosis by Anthrax Lethal Factor Through p38 MAP Kinase Inhibition. Published online August 29 2002; 10.1126/Science.1073163.

## Receptor Variant that Confers Enhanced Immune Function is a Marker for Atherosclerosis

*Background:* The ability to mount a prominent inflammatory response to a bacterial challenge confers an advantage in innate immune defense; however, the effects of intravascular inflammation may lead to increased risk of atherosclerosis later in life. The focus of this study was to determine whether genetic variants in the toll-like receptor 4 (TLR4) that confer differences in the inflammatory response due to bacterial lipopolysaccharide are related to the development of atherosclerosis. The hypothesis tested was that efficient immune defense offers an early advantage but at a cost of chronic vascular damage in later years.

*Advance:* An epidemiologic study was carried out in 810 persons in which the team screened for TLR4 polymorphisms. The extent and progression of atherosclerosis was also assessed. Fifty-five individuals were found to have a particular TLR4 polymorphism (Asp299Gly). These individuals had lower levels of certain proinflammatory cytokines and other inflammatory agents. While these subjects were more susceptible to severe bacterial infections, they had an almost 50 percent reduction in the risk carotid arterial atherosclerosis.

*Implications:* The polymorphism identified in this study decreases receptor signaling and diminishes the inflammatory response to gram-negative bacteria along with decreasing the risk of atherosclerosis. This study provides further evidence that an efficient innate immune defense against bacteria is associated with long-term intravascular inflammatory stress leading to the development of atherosclerosis. Since many environmental agents produce inflammatory responses, these results may provide insights into the mechanisms of insult and injury after exposure.

Kiechl S, Lorenz E, Reindl M, Wiedermann CJ, Oberhollenzer F, Bonara E, Williet J, Schwartz DA: Toll-like receptor 4 polymorphisms and atherogenesis. N Engl J Med 347(3): 185-192, 2002.

**Deafness, Retardation, and Attention Deficit Disorder – New Insight**

*Background:* The thyroid is a small, butterfly-shaped gland just below the Adam's apple. It plays an important role in controlling the body's metabolism, telling the body how fast to work and use energy. Thyroid hormones are dependent on iodine; iodine-deficient diets have been known to cause deafness and severe brain malformation and retardation, called cretinism, by preventing thyroid hormone synthesis. More recently less severe disruptions of thyroid signaling by mutations in the receptor proteins, or by environmental toxins that block thyroid hormone transport, have been suggested in increases in the incidence of attention deficit disorder in children. Thus, thyroid hormone is essential for normal human brain development, but the molecular mechanism of its action on developing nerve cells was unknown.

*Advance:* The majority of hormones act on target cells through one of two distinct mechanisms: by stimulating G proteins at the cell surface or by regulating gene activity inside the cell in the nucleus. Most scientists have believed that thyroid hormone acts exclusively in the cell nucleus to regulate gene expression. However, researchers recently discovered a new G protein-dependent mechanism for potassium channel protein stimulation by thyroid hormone. They demonstrated that thyroid hormone stimulates potassium channel proteins through a novel signal transduction cascade involving two proteins, PI3 kinase and the Rac GTPase. Rac and potassium channels were already known to be essential for normal neurite outgrowth in the developing brain, but the hormonal mechanisms controlling their activity during development were unknown. Potasssium channels are also required for normal sensory cell development in the inner ear. Thus, the discovery of Rac-dependent stimulation of potassium channels by thyroid hormone provides the first potential molecular explanation for several neurological disorders in humans with thyroid hormone deficiency produced by inadequate diet or by environmental toxicants.

*Implications:* Researchers have discovered a new molecular mechanism for thyroid hormone action, which has important implications for several neurological disorders in humans with thyroid hormone deficiency. It also provides new impetus for identifying industrial and agricultural chemicals, such as the halogenated aromatic hydrocarbons and pesticides, that disrupt thyroid hormone signaling.

Storey NM, O'Bryan JP, Armstrong DL: Rac and rho mediate opposing hormonal regulation of the ether-a-go-go-related potassium channel. Curr Biol 12: 27-33, 2002.

## Cigarette Smoke and High Cholesterol Increase Mitochondrial Damage in Cardiovascular Tissues

*Background:* A growing body of scientific evidence indicates that atherosclerotic lesions, the plaques that lead to hardening of the arteries and cardiac artery blockages, result from oxidative stress caused by metabolic defects and environmental exposures. Exposure to secondhand smoke (SHS) is considered a risk factor for heart disease and it has been linked to decreased blood-levels of antioxidants such as vitamins E and C, increased lipid peroxidation, and increased rates of plaque formation.

The generation of reactive oxygen and nitrogen species causes injury to the mitochondria, the energy-producing components of the cell. This mitochondrial injury can take numerous forms, ranging from mitochondrial DNA (mtDNA) damage, decreased adenine nucleotide translocator (ANT) activity, to changes in mitochondrial proteins. While the mtDNA encodes genes necessary for oxidative phosphorylation, the ANT enzyme moves adenine nucleotides across the inner mitochondrial membrane, and thus, both processes are essential for energy production by the mitochondrion, its principal job in the cell. Therefore, oxidative stress may cause mitochondrial damage that could impact a variety of cellular functions including energy production and cell signaling.

*Advance:* These investigators used a mouse model of SHS exposure and a transgenic mouse model of high cholesterol to determine whether SHS and high cholesterol can cause mitochondrial damage in cardiovascular tissues. The results show that both SHS and elevated cholesterol were associated with significantly increased mtDNA damage and protein nitration. Tobacco smoke exposure also caused decreased activities of certain mitochondrial enzymes. SHS and high cholesterol together resulted in increased plaque formation and even greater levels of mitochondrial damage.

*Implications:* The finding reported by these investigators coincide with present theories that oxidative stress mediates cardiovascular disease by causing mitochondrial damage and dysfunction. These changes ultimately lead to decreased cellular energy production and cellular dysfunction which are important early events in cardiovascular disease.

Knight-Lozano CA, Young CG, Burow DL, Hu ZY, Uyeminami D, Pinkerton KE, Ischiropoulos H, Ballinger SW: Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues. Circulation 105(7):849-854, 2002.

## Genetic Subgroups Associated with Blood Pressure Response to the DASH Diet

*Background:* Nonpharmacologic approaches to treating high blood pressure, such as diet, can be effective in reducing a person's risk of stroke or other cardiovascular disease. Previous studies have shown that the angiotensinogen (ANG) genotype is associated with development of high blood pressure and that it may influence a person's blood pressure response to nonpharmacologic therapy.

*Advance:* Researchers found that the ANG genotype is associated with blood pressure response to the Dietary Approaches to Stop Hypertension (DASH) diet. The DASH diet is rich in fruits, vegetables, and lowfat dairy foods, and limited in total and saturated fat and in red meat, sweets, and sugar-containing drinks. The blood pressure-lowering effects of the DASH diet were greatest for persons with the AA genotype of ANG, which is the same genotype previously associated with excess risk of high blood pressure.

*Implications:* These findings provide preliminary evidence that persons with a genotype that predisposes them to developing high blood pressure may also be more responsive to dietary interventions to lower their blood pressure. Additional research on other gene-intervention interactions is needed to identify persons who will most likely benefit from various high blood pressure treatments. Clinicians could then develop treatment plans specific to each person's genotype to achieve the most effective treatment for lowering blood pressure and reducing the risk of stroke and other cardiovascular disease.

Svetkey LP, Moore TJ, Simons-Morton DG, et al.: Angiotensinogen genotype and blood pressure response in the dietary approaches to stop hypertension (DASH) study. Journal of Hypertension 19(11): 1949-1956, 2001.

## New Gene Discovered that is Associated with Blood Triglyceride Levels

*Background:* Despite dramatic declines in the death rate for coronary heart disease (CHD), it remains the leading cause of death in the United States. Research has shown that blood levels of triglycerides, a type of fat, are independently associated with risk of CHD. Factors that determine blood triglyceride levels are not well understood.

*Advance:* A study supported by NIH's Programs for Genomic Applications recently identified a new gene (called the APOAV gene) that codes for a previously unknown protein. Researchers showed that mice without the APOAV gene had blood triglyceride levels four times those of normal mice, whereas mice with extra copies of the APOAV gene had triglyceride levels one-third those of normal mice. Using the latest genetic information and state-of-the-art techniques, researchers were quickly able to find the corresponding human gene, as well as variations of the gene (i.e., gene variants). Two studies in humans yielded additional insight – variants of the APOAV gene were associated with higher levels of triglycerides, independent of diet.

*Implications:* These findings suggest that screening individuals for APOAV variants could become a valuable diagnostic tool for identifying individuals likely to develop high blood triglyceride levels and CHD. With this information, clinicians would be able to advise their patients on lifestyle changes to slow, or even prevent, the development of CHD. Taking their successful research a step further, this same group of researchers is now collaborating with investigators from the NIH-supported Family Blood Pressure Program to test the association between variants of the APOAV gene and triglyceride levels in participants in that program, many of whom are from racial/ethnic minority groups who experience disproportionately high rates of CHD.

Pennacchio LA, Olivier M, Hubacek JA, et al.: An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 294: 169-173, 2001.

## Evidence of Angiogenesis in Primary Pulmonary Hypertension

*Background:* Primary pulmonary hypertension (PPH) is a debilitating disease of unknown cause that is characterized by progressive elevation of pulmonary artery pressure and leads to right-heart failure and death. The highest incidence of PPH is in women in their third and fourth decades of life. Long-term therapy with prostacyclin has improved survival and quality of life, and other promising treatment options are being evaluated. The lungs of patients with PPH contain unique structures, called plexiform lesions, which are characterized by cells that pile up and cluster within the blood vessels, completely obstructing them.

*Advance:* Investigators, using microarray technology to analyze DNA patterns, discovered a distinctive pattern of gene expression and cell growth behavior that characterizes PPH lung tissue. Results showed that plexiform lesions isolated from lungs of PPH patients produce vascular endothelial growth factor (VEGF) at high amounts, which causes abnormal growth of blood vessels by a process called angiogenesis. They also produce high amounts of a membrane protein found in endothelial and smooth muscle cells that is important in cell signaling and transport.

*Implications:* The concept that abnormal proliferation and disorderly growth of endothelial cells within plexiform lesions occurs in PPH due to altered gene expression opens the way for new studies to investigate the causes of PPH and to identify new treatment strategies. Testing of anti-angiogenesis or anti-cancer agents may lead to more effective treatments or even a cure for PPH.

Tuder RM, Chacon M, Alger L, et al.: Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. Journal of Pathology 195: 367-374, 2001.

## White Blood Cells Play an Important Role in Crises Caused by Red Blood Cell Sickling

*Background:* Sickle cell disease is characterized by defective red blood cells that tend to assume a distorted and rigid form. When the cells clog blood vessels, the result is severe pain and organ damage. We do not understand fully the step-by-step process by which blockage occurs, but evidence indicates that sickle red cells, white blood cells, and blood vessel walls are all abnormally sticky in sickle cell disease patients.

*Advance:* Scientists monitoring blood flow in mouse models of sickle cell disease observed that for the most part, it was the white blood cells, rather than the red blood cells, that adhered to the blood vessel walls. Although the sickled red blood cells played an important role in blocking blood flow, their primary interactions were with the white blood cells. In addition, when the experiment was performed in a mouse that was incapable of producing adhesion molecules on the blood vessel walls, white blood cells did not adhere to the walls, and blockage did not occur.

*Implications:* Recognition of white blood cell adhesion to blood vessels and sickle red blood cell adhesion to white cells as key events that cause vessel blockage in sickle cell disease suggests approaches to interfere with adhesion processes may have potential as therapies for sickle cell disease.

Turhan A, Weiss LA, Mohandas N, et al.: Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. Proc Nat Acad Sci 99(5): 3047-3051, 2002.

## New Evidence of Cardiac Stem Cells

*Background:* Until recently, damage to the heart was thought to be irreversible. It was believed that the body could not grow new cells to replace those that had died because of a heart attack or other heart disease. However, researchers have now challenged this idea.

*Advance:* Investigators recently discovered evidence of the existence of cardiac stem cells – that is, cells that in turn give rise to other cell types that have a variety of functions in the heart. The investigators performed postmortem examinations of eight female hearts that had been transplanted into men. Using the male Y chromosome as a marker, they found evidence of male cells with stem cell characteristics located within the (female) donor hearts. They also found that these cells differentiated into both heart muscle cells and blood vessels that grew rapidly in the new hearts after transplant. Investigators are not sure whether the male Y chromosome stem cells migrated from remnants of the male heart or from other locations, such as the bone marrow. In addition, they found a high number of cells in the donor hearts with stem cell characteristics but no male Y chromosome, suggesting that cardiac stem cells reside in the heart.

*Implications:* These results are the first strong evidence for the existence of cardiac stem cells. When combined with previous research, these results raise the possibility that some day treatments for heart attacks may be available that use the body's own ability to grow heart cells.

Quaini F, Urbanek K, Beltrami AP, et al.: Chimerism of the transplanted heart. New Engl J Med 346(1): 5-15, 2002.

## Researchers Offer Alternative Explanation for Adult Stem Cells' Apparent Plasticity

*Background:* Stem cells can make copies of themselves or differentiate into a variety of tissue-specific cells. For example, researchers have known for years that blood stem cells can make more stem cells or differentiate into red blood cells, white blood cells, and platelets. Last year, researchers also reported that blood stem cells from adults could transform into cardiac muscle cells and other cell types. This ability of adult stem cells to acquire the properties of cells in another tissue (i.e., the stem cells' "plasticity") excited the scientific community because of its therapeutic potential. If the plasticity of adult stem cells could be enhanced so that the cells transdifferentiate into other tissues, they could be used to treat many diseases, including those of the heart, lung, and blood.

*Advance:* Researchers looking to enhance the capacity of adult stem cells to transdifferentiate observed that mechanisms other than stem cell plasticity may be at work when stem cells take on the properties of cells in other tissues. They observed spontaneous fusion between mouse embryonic stem cells and mouse bone marrow cells in laboratory dishes; the resultant cells were indistinguishable from the transdifferentiated cells reported last year, at least by certain laboratory measurements.

*Implications:* This work and a similar study fusing mouse brain cells with mouse embryonic stem cells suggest that stem cell fusion may constitute a second mechanism by which stem cells can build healthy new tissues, and that researchers must consider spontaneous cell fusion when interpreting their results.

Terada N, Hamazaki T, Oka M, et al.: Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416: 542-545, 2002.

## Scientists Have Located Genes in Mice that Play a Role in Heart Failure Survival

*Background:* Heart failure is a significant public health problem. It can develop slowly, often over years, as the heart gradually loses its ability to pump enough blood through the body. Individual differences in disease progression and response to treatment remain a challenge to treating patients with heart failure.

*Advance:* Using a mouse model of heart failure, researchers recently determined specific locations on the chromosomes where genes that affect heart failure survival and cardiac function are found. The studies identified two locations (found on chromosome 2 and chromosome 3) that are significantly linked to survival. The location on chromosome 3 was also linked to alterations in cardiac function.

*Implications:* Having identified the chromosomal locations of genes that modify susceptibility to heart failure in an animal model, researchers will now focus on identifying the particular genes at those locations. This approach may allow the rapid identification of genes that play a role in heart failure survival in humans. If the genetic factors that play a significant role in modifying disease progression and outcome in heart failure patients can be identified, it may someday be possible to develop therapies that are better tailored to individual patients.

Suzuki M, Carlson KM, Marchuk DA, Rockman HA: Genetic modifier loci affecting survival and cardiac function in murine dilated cardiomyopathy. Circulation 105: 1824-1829, 2002.

## New Link Found Between Viral Infection and Asthma

*Background:* The traditional view of asthma causation is that an exaggerated immune cell response to allergic and nonallergic stimuli predisposes to the disease. A new alternative hypothesis suggests that a key to the development of asthma is a defect in the cells that line the lungs, called epithelial cells, rather than in the immune cells.

*Advance:* Investigators found that in mouse models a viral infection of the bronchioles (small tubular airways in the lungs) triggered chronic asthma-like symptoms, such as airway hyperreactivity, epithelial thickening, and mucus production, which lasted at least a year after infection. In contrast to viral infection, exposing the mice to allergens caused only a short-term induction of asthma symptoms which quickly resolved.

*Implications:* Further studies in humans and on mouse models could lead to important new strategies to prevent asthma in children who are at high risk for the disease.

Walter MJ, Morton JD, Kajiwara N, et al.: Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. J Clin Invest 110(2): 165-175, 2002.

## Study Finds Dramatic Decline in Physical Activity Among Black and White Girls During Adolescence

*Background:* Obesity in children and adolescents has been increasing, particularly over the past 20 years. Low levels of physical activity may be a reason for this trend. Understanding factors related to the decline in physical activity in adolescence may help guide future intervention efforts to prevent this decline and perhaps prevent the rising tide of obesity.

*Advance:* A large study tracked the exercise habits of adolescent girls, starting when they were nine or ten years old and ending ten years later. It found that by the time the girls were 16 or 17 years old, 56 percent of black girls and 31 percent of white girls reported no habitual leisure-time physical activity. For girls of either race, body mass index (a measure of body weight adjusted for height) at the beginning of the study was directly associated with declines in activity levels, with the heaviest girls experiencing the greatest declines in physical activity. Additional factors that predicted a decline in physical activity included pregnancy, smoking, and lower levels of parental education.

*Implications:* These findings confirm that a substantial decline in physical activity occurs during adolescence in girls, and that this decline is greater in black girls than white girls. Intervention programs to increase physical activity in girls should start before ages 12-13, when the precipitous drop in physical activity occurs, and preferably at ages younger than 9-10. The risk factors identified in this study will help prioritize education resources and guide prevention efforts to reduce the prevalence of obesity in children and adolescents.

Kimm SY, Glynn NW, Kriska AM, et al.: Decline in physical activity in black girls and white girls during adolescence. New Engl J Med 347(10): 709-715, 2002.

## Stress Linked to Asthma Attacks

*Background:* Over 15 million Americans have asthma. Its prevalence has doubled in the past 20 years and is still increasing, particularly among young children. Asthma attacks can be life-threatening and are a leading cause of absenteeism from school and work. Despite all that we know about asthma, the role of stress in causing asthma attacks is not well understood.

*Advance:* In the first of two significant studies, researchers found that perceived stress experienced by caregivers was correlated with subsequent episodes of wheezing in their infants. This effect was observed regardless of other factors that might contribute to wheezing episodes, such as caregiver smoking, exposure to allergens, or lower respiratory infections. A second study, involving otherwise healthy college students with mild allergic asthma, found that the stress related to taking exams caused increased inflammation in the airways and heightened airway reactivity in response to exposure to allergens, such as ragweed, cat dander, or house dust mite. These findings suggest that stress may play a role in increasing the severity of asthma attacks.

*Implications:* The results from the study of caregivers and infants indicates that family stress can lead to an increased risk of wheezing, perhaps by influencing development of an infant's immune system. The study of students taking exams is one of the first to show a link between stress and the specific biological events leading to asthma symptoms. If researchers can unravel the mechanisms by which stress shapes the immune system or induces asthma attacks, then improved methods can be developed to prevent attacks when a person experiences a stressful event. Additional research will be necessary to examine the effects of different kinds of stress on asthma.

Wright RJ, Cohen S, Carey V, et al.: Parental stress as a predictor of wheezing in infancy: a prospective birth-cohort study. Am J Respir Crit Care Med 165; 358-365, 2002.

Liu LY, Coe CL, Swenson CA, et al.: School examinations enhance airway inflammation to antigen challenge. Am J Respir Crit Care Med 165; 1062-1067, 2002.

## Sarcoidosis Study Finds Factors Influencing Susceptibility and Location

*Background:* Sarcoidosis is a systemic disease involving organ systems throughout the body, in which normal tissue is invaded by pockets of inflammatory cells called granuloma. Its cause is unknown, but appears to involve a genetic predisposition and environmental exposure. It is estimated that as many as 134,000 Americans have sarcoidosis, and as many as 29,000 new cases may occur each year.

*Advance:* Investigators working on A Case Control Etiologic Study of Sarcoidosis (ACCESS), which included a large group of patients and their relatives, found that parents and siblings of sarcoidosis patients are nearly six times more likely to get sarcoidosis than someone in the general population. Data also indicate that white patients are more likely to have relatives with sarcoidosis than black patients. ACCESS researchers also found that age, gender, and race influence organ involvement: sarcoidosis that involves an organ other than the lung (extrapulmonary) is more likely to occur in African American patients, younger patients, and female patients.

*Implications:* Genetic studies in family members of sarcoidosis patients should help identify genes associated with susceptibility to sarcoidosis. The research also provides a clearer picture of how sarcoidosis affects different types of patients and different organs. This should permit clinicians to better identify which organs are most likely to be affected, thereby improving monitoring and treatment.

Rybicki BA, Iannuzzi MC, Frederick MM, et al. Familial aggregation of sarcoidosis. Am J Respir Crit Care Med 164: 2085-2091, 2001.

Baughman RP, Teirstein AS, Judson MA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 164: 1885-1889, 2001.

## Gene Transfer Provides Long-Term Protection from Heart Damage in Rats

*Background:* Single or intermittent periods of myocardial ischemia (insufficient supply of blood to the heart usually due to blockage in the coronary arteries) and reperfusion (subsequent restoration of blood flow) – such as occur with heart attacks – can lead to heart tissue damage. Of the estimated 1.1 million Americans who suffer heart attacks each year, nearly half have experienced a prior attack. Therefore, a therapy that could be administered to heart attack survivors to reduce or prevent heart damage in case of a second heart attack has the potential to save many lives.

*Advance:* Researchers experimented with a new gene therapy technique for providing long-term protection to the heart from ischemic damage. Eight weeks before they induced ischemia/reperfusion in the hearts of rats (by tying off a coronary artery and subsequently releasing it), researchers delivered the human gene for heme oxygenase-1, which is known to protect cells, into the normal rat hearts. Results showed that this gene transfer reduced the size of subsequent heart damage by 75 percent compared with control animals that did not receive the gene.

*Implications:* This study shows that gene therapy, even when administered well before ischemia occurs, can protect the hearts of animals from damage. If this technique can be used successfully in humans, it could be beneficial as a preventive therapy for patients who have already had a heart attack and are, therefore, at high risk of suffering a subsequent attack.

Melo LG, Agrawal R, Zhang L, etal.: Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene. Circulation 105: 602-607, 2002.

## New Form of Aspirin Blocks Restenosis in Rats

*Background:* Coronary angioplasty (also called balloon angioplasty) is often used to treat individuals with severe blockages in their coronary arteries. A tiny balloon, inserted into the narrowed coronary artery, is inflated to open up the artery and increase blood flow. Although the procedure is initially successful in most patients, within 6 months 35 percent of them require a follow-up procedure because of a restenosis, or renarrowing, in the affected arteries.

*Advance:* Researchers recently compared the effects of treatment with an aspirin modified to release nitric oxide (NO) versus ordinary aspirin in adult and elderly rats with restenosis. They found that NO-releasing-aspirin given several days before and after angioplasty significantly reduced restenosis, while ordinary aspirin had no substantial effect. The beneficial effect was especially pronounced in the elderly rats.

*Implications:* These findings indicate that an NO-releasing compound might be an effective drug for inhibiting restenosis following balloon angioplasty. Testing NO-releasing-aspirin in a wider range of animal models, followed by studies in human patients, are necessary remaining steps toward determining its potential clinical use.

Napoli C, Aldini G, Wallace JL, et al.: Efficacy and age-related effects of nitric oxide-releasing aspirin on experimental restenosis. Proc Nat Acad Sci 99(3): 1689-1694, 2002.

## Strategies for Protecting the Brain During Heart Surgery Hold Promise for Children

*Background:* Neurological problems are a troubling complication for many of the 20,000 children who undergo surgery for congenital heart problems each year in the United States.

*Advance:* Researchers have been making considerable progress in determining intraoperative conditions that are less likely to lead to brain damage. Although it was thought that diluting the blood and thereby lowering hematocrit (the proportion of red blood cells in the circulation) might be beneficial, researchers using a piglet model found the opposite to be true. They also found that a specific acid-base management strategy for keeping the blood components balanced as the blood goes through a bypass machine improves patient outcome.

*Implications:* These findings have important implications for reducing neurological complications of congenital heart surgery, thereby improving patient outcome.

Sakamoto T, Zurakowski D, Duebener LF, et al.: Combination of alpha-stat strategy and hemodilution exacerbates neurologic injury in a survival piglet model with deep hypothermic circulatory arrest. Ann Thorac Surg 73: 180-190, 2002.

## Cardiac Hypertrophy May Not be Necessary for Preserving Heart Function in Heart Failure Patients

*Background:* Heart failure is often caused by chronic pressure overload such as that brought on by longstanding hypertension. It usually develops slowly, as the heart gradually loses its ability to pump sufficient quantities of blood through the body. Scientists have long thought that cardiac hypertrophy, a thickening of the heart muscle fibers, develops to compensate for chronic pressure overload, and that the hypertrophy helps maintain normal cardiac function and prevent the development of heart failure. Recent data, however, have cast doubt on this theory.

*Advance:* To test whether cardiac hypertrophy is necessary to preserve cardiac function in response to pressure overload, researchers used genetically engineered mice designed to develop much less hypertrophy in response to pressure overload than normal mice. The genetically altered mice showed little or no deterioration in cardiac function in response to pressure overload despite their diminished ability to develop hypertrophy. Moreover, researchers found that normal mice, that developed hypertrophy in response to exposure to increased pressure overload, showed progressive decline in cardiac function.

*Implications:* Contrary to previous thought, these data suggest that cardiac hypertrophy may not be necessary to preserve cardiac function in situations of disease or injury. Studies in larger animal models are now needed to increase our understanding of the role hypertrophy plays in the development of heart failure. Additional knowledge about hypertrophy will help scientists to develop better treatment strategies for preventing or slowing the development of heart failure.

Esposito G, Rapacciuolo A, Prasad SVN, et al.: Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress. Circulation 105: 85-92, 2002.

## The Role of Stress in the Deregulation of Blood Clotting May Explain its Role in Cardiovascular Disease

*Background:* Many heart attacks and strokes originate from blood clots in the arteries. Physical and emotional stress are thought to be implicated in these events, but the mechanisms by which stress increases the risk of blood clots are unclear.

*Advance:* Researchers found that expression of the gene for plasminogen activator inhibitor (PAI-1), a major inhibitor of a system that dissolves blood clots, increases dramatically in mice that are subjected to stress. Moreover, the increase was more pronounced in old and obese mice.

*Implications:* These observations provide a molecular explanation of how stress may increase the risk of heart attacks and strokes and indicate that stress may combine with age and obesity to cause cardiovascular disease.

Yamamoto K, Takeshita K, Shimokawa T, et al.: Plasminogen activator inhibitor-1 is a major stress-regulated gene: implications for stress-induced thrombosis in aged individuals. Proc Nat Acad Sci 99(2): 890-895, 2002.

## Can the Same Strategy Prevent Two Different Types of Vascular Disease?

*Background:* Much is known about factors that increase risk of cardiovascular events such as heart attack and stroke, which result from blood clot formation (thrombosis) in arteries. However, factors leading to problems such as pulmonary embolism, which result from clots in veins, are less well understood.

*Advance:* A recent study found that some risk factors for arterial thrombosis – smoking, lipid levels, blood pressure – are not correlated with venous thrombosis. However, two conditions – obesity and diabetes – are linked to clots in both locations.

*Implications:* The identification of new risk factors for venous thrombosis provides grounds for concern and additional incentive to address the growing prevalence of obesity and diabetes in the United States.

Tsai AW, Cushman M, Rosamond WD, et al.: Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. Arch Intern Med 162: 1182-1189, 2002.

## Failure of AIDS Vaccine in Rhesus Monkey Provides Key Information for Better Vaccine Design

*Background:* Simian-human immunodeficiency virus (SHIV) is a genetically engineered hybrid monkey-human virus that mimics human immunodeficiency virus (HIV) infection and causes a serious Acquired Immunodeficiency Syndrome (AIDS)-like illness in monkeys. Previous studies have shown that immunizing rhesus monkeys with a vaccine containing several genes from SHIV can control viral replication in these animals and protect them from disease progression once they become infected with SHIV. This protection occurs because the vaccine stimulates potent cytotoxic T lymphocytes (CTLs), or "killer" T cells, to respond to the virus, effectively controlling the level of infection. However, previous studies of HIV-infected people and simian immunodeficiency virus (SIV)-infected monkeys indicated that some viruses may mutate, thereby escaping recognition and destruction by CTLs.

*Advance:* In a study of rhesus monkeys that were vaccinated and subsequently infected with SHIV, NIH-supported investigators recently reported that one of the eight animals whose immune system had been controlling the virus showed an increase in virus replication starting at 24 weeks after infection. Soon after, the animal's immune system exhibited a severe loss of CD4+ T cells, immune cells that are depleted in humans who have AIDS. The monkey developed an AIDS-like condition, and died by week 52. The loss of virus control was associated with a mutation in a CTL target sequence ("epitope") contained within a viral protein. Viruses containing the mutation were no longer susceptible to control by the CTLs that had targeted that epitope and were therefore able to replicate to a level sufficient to cause disease. Although there may have been other mutations that were not detected, it is possible that this single mutation led to the sudden increase in viral replication and disease progression.

*Implications:* This study points out important limitations that researchers will need to overcome to develop more effective AIDS vaccines, particularly those that produce cellular immune responses involving CTLs to control virus replication after infection. In the future, researchers will need to design vaccines that can elicit the greatest possible breadth of CTL responses, each of which targets a different viral epitope, so that escape from one CTL target will not allow the virus to replicate. In addition, researchers will need to develop vaccines that reduce virus replication to the lowest possible levels after infection to minimize the chance that viral mutations will occur.

Barouch DH, Kunstman J, Kuroda MJ, Schmitz JE, Santra S, Peyerl FW, Krivulka GR, Beaudry K, Lifton MA, Gorgone DA, Montefiori DC, Lewis MG, Wolinsky SM, Letvin NL: Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature 415: 335-339, 2002.

## Researchers Discover How Anthrax's Killer Toxin Enters Cells

*Background:* Anthrax poses a significant threat as an agent of biological warfare and terrorism. The anthrax bacterium causes illness and death largely by releasing a potent toxin that enters and kills cells and damages organs. Few people survive when the microbe becomes widespread in the body. This is because the toxin remains active in the bloodstream for several days, even if antibiotics kill the bacteria that are producing the toxin. The anthrax toxin is composed of three distinct molecules that work together: edema factor (EF) and lethal factor (LF), which enter cells and destroy them from the inside or keep them from working, and protective antigen (PA), which carries EF and LF into cells so these factors can do their damage. The PA component of the toxin unit attaches to the surface of many human cells. Once the toxin is attached, PA acts like a hypodermic needle, injecting EF and LF into the cell. Until now, researchers had not identified the cell surface molecule to which PA binds to gain entry into the interior of the cell and cause damage.

*Advance:* Through genetic analysis, NIH-supported scientists have identified a receptor, a protein normally found on the outside of cells in the body, to which the PA attaches. The receptor, termed the anthrax toxin receptor (ATR), allows anthrax toxin to gain entry into those cells and destroy them. In addition to identifying the ATR, researchers identified the specific region on ATR to which the toxin attaches. With this information, they were able to produce a shortened, free-floating version of the receptor that contained the toxin-binding region. When the scientists mixed this free-floating receptor fragment with animal cells and anthrax toxin in a test tube, the receptor fragment acted like a sponge or decoy and absorbed the anthrax toxin before it could attach to the cells. As a result, the cells were completely protected from destruction.

*Implications:* These findings hold promise for developing new approaches for treating anthrax disease by targeting the toxin itself. Now that researchers have identified the anthrax toxin receptor and know what it looks like, they can screen large numbers of smaller molecules that have the potential to bind to the toxin and prevent it from docking onto ATR and entering and destroying cells.

Bradley KA, Mogridge J, Mourez M, Collier RJ, Young JAT: Identification of the cellular receptor for anthrax toxin. Nature 414: 225-229, 2001.

## HIV Selectively Suppresses Anti-HIV Defense Cells

*Background:* Human immunodeficiency virus (HIV), the virus that causes Acquired Immunodeficiency Syndrome, attacks the immune system and leaves the body vulnerable to a variety of life-threatening illnesses and cancers. Crucial immune cells called CD4+ T cells are disabled and killed during the typical course of infection. These cells, sometimes referred to as "T-helper cells," play a central role in the immune response, signaling other cells in the immune system to perform their special functions. During HIV infection, the number of CD4+ T cells in a person's blood progressively declines either through their destruction or decreased production. When the CD4+ T-cell count falls below 200 per milliliter, the HIV-infected person becomes particularly vulnerable to the opportunistic infections and cancers that typify AIDS, the end stage of HIV disease. For years, researchers have known that the immune system does not produce a strong CD4+ T-cell response against HIV. They have postulated that HIV preferentially infects HIV-specific CD4+ cells, that is, the very cells that are specifically designed to fight the virus.

*Advance:* NIH scientists isolated CD4+ T cells from 12 HIV-infected individuals and divided them into subgroups: HIV-fighting cells, cells programmed to fight cytomegalovirus (a herpes-type virus that is common in people infected with HIV), and a "mixed" group. The scientists found that CD4+ T cells programmed to fight HIV are two to five times more likely to be infected with HIV than CD4+ T cells programmed to combat other disease-causing organisms in people at all stages of HIV disease. In an attempt to learn whether the virus attacks both young, or naïve, helper T cells that are present in the early stages of HIV infection as well as mature helper T cells present in later, chronic infection, the scientists also examined the cells of four HIV-positive individuals who were past the early stages of HIV infection and undergoing structured treatment interruptions of their antiretroviral drug therapy (periods of drug treatment interspersed with periods of discontinuation of therapy). Because these individuals were past the early stage of their infection, all their naïve helper T cells had matured. They found that HIV continuously and preferentially infects mature HIV-specific helper T cells as they try to fight off the virus.

*Implications:* The results of this study confirm for the first time what researchers have suspected: HIV selectively disables the immune system's response against the virus by disproportionately infecting the very cells designed to fight it at all stages of infection. This provides a potential explanation for the loss of HIV-specific CD4+ T-cell responses in infected individuals. The results also provide a cautionary note for physicians about HIV treatment approaches involving scheduled interruptions in drug treatment that allow the virus levels to rebound. Such an approach may result in long-term damage of the immune system's ability to fight off HIV by increasing the proportion of HIV-specific CD4+ T cells infected with the virus. The findings should also aid in developing a more effective HIV vaccine. An effective vaccine must induce a strong T-cell response against HIV.

Douek DC, Brenchley JM, Betts MR, Ambrozak D, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kunstman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA: HIV preferentially infects HIV-specific CD4+ T cells. Nature 417: 95-98, 2002.

## Researchers Identify Novel Asthma Susceptibility Genes

*Background:* Asthma is a chronic respiratory disorder characterized by intermittent difficulty in breathing, wheezing, coughing and increased mucus production. The lungs of patients with asthma are hyperreactive to allergy-causing substances, such as dust mites and pollen, exercise, and breathing cold air and tobacco smoke. Asthma prevalence has increased in industrialized societies, and epidemiological studies suggest that the increase may be the result of improved hygiene and reduced frequency of infections such as tuberculosis and hepatitis A. It is believed that asthma results from complex interactions between multiple genetic and environmental factors, but the specific genes involved in asthma susceptibility have not been conclusively identified.

*Advance:* Using a novel mouse model that mimics human asthma, NIH-supported scientists have identified a single chromosomal region called *Tapr*, which regulates the function of immune cells associated with experimental asthma. Within the *Tapr* region, the investigators identified a family of genes, *Tim,* that is associated with the development of airway hyperreactivity and the regulation of immune cell activity. Genetic variations in two members of the *Tim* family, *Tim1* and *Tim3*, correlated with the development of airway hyperreactivity, and the researchers found additional evidence indicating that *Tim1* plays a major role in asthma susceptibility. Interestingly, the protein encoded by the *Tim1* gene is a relative of the protein to which the hepatitis A virus attaches in human cells.

*Implications:* This study suggests that the *Tim* gene family, and in particular *Tim1*, plays an important role in the regulation of asthma development. Future studies may show more definitively whether this gene family is involved in human asthma susceptibility. In addition, this mouse model may help researchers develop ways to prevent asthma, since the findings also suggest a link between the *Tim* gene family and the protective effect of hepatitis A infections against the development of asthma.

McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, Freeman GJ, Umetsu DT, DeKruyff RH: Identification of *Tapr* (an airway hyperreactivity regulatory locus) and the linked *Tim* gene family. Nat Immunol 2(12): 1109-1116, 2001.

## A Single Gene Change Aided the Emergence of Bubonic Plague

*Background:* The bubonic plague, or "Black Death," killed one-fourth of Europe's population in the 14th century. Yet *Yersinia pestis*, the bacterium that causes the plague, once existed in a form that produced only a mild intestinal illness that was acquired through contaminated food or water. Some relatively recent evolutionary adaptation by *Y. pestis* enabled it to become a much more deadly microbe. One key difference between the plague-causing *Y. pestis* and its more benign ancestors is its ability to infect humans through the bite of a flea. In fact, the transmission of *Y. pestis* through a blood-feeding insect is unique among all closely related but less harmful bacteria that live and multiply in the human digestive tract.

*Advance:* Investigators at NIH recently discovered a single gene that appears to have played a crucial role in the evolution of *Y. pestis* into a lethal bacterium. Previous research had indicated that *Y. pestis* acquired a gene that produces an enzyme called phospholipase D (PLD) through transfer of genetic material from an unrelated organism. In the recent study, NIH investigators infected fleas with versions of *Y. pestis* that either contained or lacked the PLD gene. The researchers found that the PLD gene was required for the survival of the bacterium in the gut of the flea. By protecting *Y. pestis* from being destroyed in the gut, the PLD gene allowed the bacterium to infect fleas and opened up the new route of its transmission to humans through fleabites. In turn, this adaptation to transmission by a blood-feeding insect may have allowed more deadly strains of the bacteria to emerge.

*Implications:* These discoveries help explain the forces behind the emergence of a disease that once devastated the human population. The findings also reveal the profound effect that the change of a single gene can have on the evolution of a disease. This knowledge is useful for understanding and fighting other infectious diseases because microbes frequently exchange genetic material and can repackage themselves in many ways to emerge as new or more deadly agents of human disease. The example of plague's emergence helps scientists understand one mechanism by which this process can occur.

Hinnebusch BJ, Rudolph AE, Cherepanov P, Dixon JE, Schwan TG, Forsberg A: Role of Yersinia murine toxin in survival of *Yersinia pestis* in the midgut of the flea vector. Science 296: 733-735, 2002.

## New Findings Provide Clues for Designing an Effective HIV/AIDS Vaccine

*Background:* Human immunodeficiency virus (HIV) vaccine research has focused increasingly on the role that immune system cells called CD8+ cytotoxic T lymphocytes (CTLs) play in protecting against infection with HIV and/or the development of Acquired Immunodeficiency Syndrome (AIDS). CTLs, also known as killer T cells, are a type of white blood cell that can kill other cells that are infected with viruses such as HIV or simian immunodeficiency virus (SIV), a virus similar to HIV that causes an AIDS-like illness in monkeys. Many researchers believe an effective vaccine against HIV will need to stimulate a rapid and strong CTL response. However, scientists do not know which of the many types of CTL responses that occur during viral infection are important for controlling replication of the virus. Furthermore, studies have established that CTL responses to HIV and SIV can promote the survival of so-called escape variants of the virus during chronic (later stage) infection. These variant forms of the virus contain mutations that allow them to elude detection and elimination by CTLs. Escape variants arise within an infected individual as the virus undergoes continual genetic changes. Whenever the immune response destroys one variant, a distinct but related variant can emerge that is resistant to that immune response, thereby perpetuating the infection.

*Advance:* Using SIV as a model for HIV infection, NIH-supported researchers showed that viral escape from CTLs is a hallmark of the acute (early) stage of SIV infection, when the virus is multiplying rapidly. The researchers found that viruses from 19 of the 21 SIV-infected monkeys they studied escaped from at least one type of CTL response during acute SIV infection. They also identified six new types of CTL responses that appeared to promote viral variation in the acute phase of infection. Notably, the CTLs that promoted the survival of escape variants during the acute phase were of a type that is thought to be particularly effective at killing virus-infected cells.

*Implications:* Because of the similarity between acute SIV and HIV infection, these findings may lead to a better understanding of how CTL responses contribute to the control of HIV and what types of CTL responses must be stimulated by a vaccine to prevent the problem of viral escape variants. The finding that specific types of CTLs promote the survival of escape variants during early SIV infection suggests that identifying analogous CTLs in HIV infection may be important for HIV vaccine development. Such CTLs may be fundamentally different from CTLs that do not promote the survival of escape variants during early infection and may be less effective at protecting against HIV.

O'Connor, DH, Allen TM, Vogel TU, Jin P, DeSouza IP, Dodds E, Dunphy EJ, Melsaether C, Moth_ B, Yamamoto H, Horton H, Wilson N, Hughes AL, Watkins DI: Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nat Med 8(5): 493-499, 2002.

## Cholera Bacteria Become More Virulent by Passing Through the Human Intestines

*Background:* Cholera is a severe and potentially deadly diarrheal disease caused by infection of the intestine with the bacterium *Vibrio cholerae*. It has a rapid onset and most often occurs in epidemics spread through contaminated water. Cholera has been very rare in industrialized nations for the last 100 years; however, the disease is still common today in other parts of the world where sanitary conditions, particularly sewage systems, are less than optimal. The challenge for public health officials is to contain cholera outbreaks in the developing world. However, information on the factors that enable the epidemic spread of cholera is limited. For example, researchers know that the water-borne cholera bacterium spreads quickly among people during outbreaks but, for reasons that have been unclear, is much less infectious when grown in the laboratory.

*Advance:* NIH-supported researchers collaborating with investigators in Bangladesh recently used studies of gene activity in *V. cholerae* isolated from stool samples to gain new insights into how the bacterium spreads so rapidly from person to person. The researchers demonstrated that passage through the human digestive tract appears to switch on key genes of the cholera bacterium, making it up to 700 times more infectious than cholera grown in the laboratory. In addition, they showed that this heightened infectivity is maintained even after the bacteria are released into the environment, a property that likely contributes to the epidemic spread of cholera. The studies of gene activity in the cholera bacterium were done using so-called gene expression profile ("gene chip") technology. This technique allows researchers to determine rapidly the activity of thousands of different genes in a particular cell, tissue, or organism. By measuring which genes are turned on or off under different conditions, researchers obtain clues about which genes (and the proteins they encode) are important in various biological processes.

*Implications:* These findings identify the process by which cholera epidemics may be spread by humans and provide insights into the bacterial genes that may be critical for this process. This information should help researchers identify new targets for antibacterial drugs to prevent cholera transmission and vaccines to prevent infection. The findings also may shed light on whether other virulent microorganisms are spread among humans in a similar manner. In addition, this study demonstrates the utility of gene expression profiling technology for deciphering a complex epidemiological problem.

Merrell DS, Butler SM, Qadri F, Dolganov NA, Alam A, Cohen MB, Calderwood SB, Schoolnik GK, Camilli A: Host-induced epidemic spread of the cholera bacterium. Nature 417: 642-645, 2002.

## Enhanced Induction of Antibody Response to an HIV Vaccine

*Background:* Studies suggest that an effective human immunodeficiency virus (HIV) vaccine will need to trigger both a cellular immune response and a strong antibody response to the virus. The cellular immune response consists primarily of cytotoxic T lymphocytes, or "killer" T cells – white blood cells that attack other cells that are infected with the virus. The antibody response is the other main type of immune response that can control foreign invaders such as viruses. It consists primarily of neutralizing antibodies that circulate in body fluids and prevent free-floating virus from infecting cells in the body. DNA vaccines are one type of potential HIV vaccine that researchers are studying for their ability to elicit both a neutralizing antibody and cytotoxic T-lymphocyte response. HIV DNA vaccines are direct injections of genes coding for specific HIV proteins. When the DNA is injected, the encoded viral proteins are produced, triggering an immune response to these "foreign" proteins. HIV DNA-based vaccines readily generate cellular immunity, but their ability to trigger strong antibody responses has been limited – particularly the response to the HIV "envelope" protein, Env.

*Advance:* NIH researchers have developed a modified form of the HIV *env* gene that produces both cellular immunity and significantly higher levels of neutralizing antibodies in animals after vaccination. The HIV *env* gene provides the genetic code to produce the Env protein, which gives rise to the two proteins that form the envelope, or outer surface, of the virus. The researchers created a series of modified *env* genes, inserted each modified gene into a larger piece of DNA, and injected the DNA into mice. They then measured the antibody and cellular immune responses to each of the modified *env* genes and identified one version of the gene, called gp140□CFI, that triggered the production of significantly higher levels of neutralizing antibodies to the HIV Env protein and maintained its ability to generate a cellular immune response to Env.

*Implications:* The results of these animal studies suggest that the gp140□CFI version of the HIV *env* gene could be used as the basis of a DNA vaccine that can trigger a neutralizing antibody response against the HIV envelope while retaining its capacity to trigger a cellular immune response to HIV. Vaccines that trigger both a cellular and antibody response to HIV are desirable because they have the features believed necessary to provide effective protection against the virus.

Chakrabarti BK, Kong W-p, Wu B-y, Yang Z-Y, Friborg J, Ling X, King SR, Montefiori DC, Nabel GJ: Modifications of the human immunodeficiency virus envelope glycoprotein enhance immunogenicity for genetic immunization. J Virol 76(11): 5357-5368, 2002.

## Scientists Discover New Influenza Virus Protein that Causes Cell Death

*Background:* The influenza A virus is a major cause of disease and death in the United States and has the potential to cause devastating epidemics. The sequence of the viral genome, which encodes (that is, contains the molecular instructions for) the proteins that make up the virus, was deciphered more than 20 years ago, making it one of the first organisms to be completely characterized genetically. Until recently, researchers believed that all of the proteins encoded by the influenza virus genome had been discovered.

*Advance:* NIH scientists discovered a new, "hidden" influenza A virus protein whose gene was lurking undetected within a gene that encodes one of the 10 known viral proteins. This new protein may kill immune cells by activating the cell's ability to self-destruct, or commit "cell suicide." The discovery, like so many others in biology, was serendipitous. The researchers were led to the new protein by experiments designed to understand how products of the influenza A virus are recognized by the immune system. The protein, named PB1-F2, has many interesting features, including localization in mitochondria, which are the cellular power plants that are intimately involved in the process of cell suicide. PB1-F2 seems to trigger cell death but only in a subset of cells that are involved in the early immune response to infection with influenza A virus.

*Implications:* NIH researchers are currently investigating whether PB1-F2 enables influenza A virus to multiply more quickly in infected individuals by disabling this important part of the immune system. They are also working to understand more about how this newly discovered viral protein achieves its effects. The team will study whether this protein might have contributed to the particularly lethal nature of the flu viruses that caused the Asian flu of 1957, the Hong Kong flu of 1968, and the Spanish flu of 1918 that killed 20 million people worldwide. This study highlights the importance of supporting basic research on infectious diseases as a pathway for unexpected scientific discoveries. As a result of basic scientific inquiry, NIH scientists uncovered information that may help explain why some forms of influenza are more deadly and persistent than others and help scientists stem future epidemics.

Chen W, Calvo PA, Malide D, Gibbs J, Schubert U, Bacik I, Basta S, O'Neill R, Schickli J, Palese P, Henklein P, Bennink JR Yewdell JW: A novel influenza A virus mitochondrial protein that induces cell death. Nat Med 7(12): 1306-1312, 2001.

## Clues into How the Immune System Fights Tuberculosis

*Background*: Tuberculosis (TB) is a contagious airborne infectious disease that attacks the respiratory system and is spread easily through coughing and sneezing. *Mycobacterium tuberculosis,* the pathogen that causes TB, kills more people than Acquired Immunodeficiency Syndrome (AIDS) and malaria combined. Approximately one-third of the world's population is infected with *M. tuberculosis*, and 1 in 10 of these individuals will likely develop active TB disease. Researchers have been studying the details of how the immune system uses different types of cells to fight acute and chronic *M. tuberculosis* infection, in particular the two immune system cells CD4+ and CD8+ T cells. The functions and manifestations of these two T-cell types are different. For example, CD4+ T cells play a major role in regulating immune reactions and are known to produce factors called cytokines that are important in controlling infections. On the other hand, CD8+ T cells are cytotoxic, or destructive, lymphocytes that destroy infected cells. However, investigators know little about the interaction between CD4+ and CD8+ cells during *M. tuberculosis* infection, specifically what other roles CD4+ T cells play in the defense against this disease.

*Advance:* During a recent study, NIH-supported researchers examined the effect of CD4+ T-cell deficiency on the development of cytokine production and cytotoxic functions of CD8+ cells in *M. tuberculosis*-infected mice. Cytokines are proteins that regulate the intensity and duration of immune responses. The researchers found that the deficiency of CD4+ T cells did not affect the $CD8^+$ T cell's ability to mature and migrate in lung and lymph node tissue, nor did it affect the cell's ability to produce cytokines following *M. tuberculosis* infection. However, researchers determined that the development of the cell-killing capacity of CD8+ T cells was impaired in mice with CD4+ T-cell deficiency. In other words, researchers demonstrated that CD4+ T cells not only contribute to protective immunity but also help CD8+ T cells destroy infected cells, thereby contributing to the control of *M. tuberculosis* infection.

*Implications:* A thorough understanding of how CD4+ T cells interact with CD8+ T cells, as well as with other cell types, to fight TB is important, especially for those individuals with weakened immune systems, such as people with human immunodeficiency virus/AIDS. These findings also may provide insight into why patients with AIDS develop TB more rapidly than healthy individuals after being infected with *M. tuberculosis*. A better understanding of interactions between CD4+ and CD8+ T cells ultimately will facilitate the development of successful vaccines and therapeutic strategies against TB.

Serbina NV, Lazarevic V, Flynn JL: $CD4^+$T cells are required for the development of cytotoxic $CD8^+$T cells during *Mycobacterium tuberculosis* infection. J Immunol 167: 6991-7000, 2001.

## Men and Women Respond Differently to Gonorrheal Infections

*Background:* Gonorrhea, caused by a bacterium called *Neisseria gonorrhoeae*, is one of the three most common sexually transmitted infections caused by bacteria. In 2000, more than 350,000 cases of gonorrhea were reported to the U.S. Centers for Disease Control and Prevention. Gonorrhea bacteria can infect the genital tract, the mouth and throat, the urethra, and the anus and rectum. The disease is unusual in that the course of infection is different in men than in women. Symptoms usually begin shortly after infection in men, whereas the development of symptoms in women is usually more protracted. In addition, women are more likely to have mild symptoms or no symptoms at all. Research has shown that during infection in men, gonorrhea bacteria invade cells that line the urethra. However, scientists did not know many details about how the bacteria get inside these cells. To learn more about this process, NIH-supported researchers developed a system that allows them to grow cells from the lining of the urethra in the laboratory and infect them with gonorrhea bacteria. Initial studies indicated that the bacteria enter urethral cells with the help of a receptor – a molecule on the outside of cells that acts as a gateway to the cell's interior.

*Advance:* Using their system of laboratory-grown cells from the lining of the male urethra, which were obtained from men undergoing prostate surgery, NIH-supported researchers uncovered new details about how gonorrhea bacteria invade these cells. The researchers showed that gonorrhea bacteria bind to a particular type of receptor molecule on the urethra cell exterior, known as the asialoglycoprotein (ASGP) receptor. They also found that a sticky molecule on the surface of the gonorrhea bacterium, which binds to the ASGP receptor, plays a key role in the microbe's ability to invade cells from the urethra. The interaction between these two molecules – one on the bacterium, and the other on the cell of the urethral lining – appears to trigger a process known as endocytosis, in which the sac-like membrane that encloses the cell folds inward to bring substances (in this case the bacterium) into the cell.

*Implications:* Such detailed information on the mechanisms by which gonorrhea bacteria interact with and invade the cells of the male urethra may lead to the development of new and more effective treatment and prevention measures. The results of this study coupled with the results from other studies suggest that gonorrhea bacteria have evolved a number of different mechanisms to adhere to and enter human cells and that these mechanisms differ between men and women. For example, another recent study showed that gonorrhea bacteria infect women through a different receptor molecule found on cells of the cervix. These two findings taken together may help explain why the symptoms of gonorrhea infection differ between men and women and suggest that the specific immune response on mucosal surfaces (which include, for example, the nasal passage, gastrointestinal tract, and urogenital lining) is an important component of protection against gonorrheal infections. Ultimately, these findings , and mayalso lead to new ways to treat and prevent the disease.

Harvey HA, Jennings MP, Campbell CA, Williams R, Apicella MA: Receptor-mediated endocytosis of *Neisseria gonorrhoeae* into primary human urethral epithelial cells: the role of the asialoglycoprotein receptor. Molecular Microbiology 42(3): 659-672, 2001.

## Viruses Help Strep Bacteria Become More Dangerous

*Background:* A common bacterium can turn into a potent killer if it picks up the right set of genes, but how and when those genes are acquired have remained poorly understood. In some cases, viruses called bacteriophages infect bacteria, capture some of the bacterial genes, and transfer those genes from one bacterium to the next. By moving genes among their bacterial "hosts," bacteriophages can create new bacterial strains with potentially deadly properties. Scientists have known about bacteriophages for some time, but these viruses have not been extensively studied for their possible indirect contributions to infectious diseases. Group A streptococcus (GAS) bacteria are common microbes that cause many different diseases, including strep throat, wound infections, toxic shock, "flesh-eating" disease, scarlet fever, rheumatic fever, and kidney ailments. One particular strain of GAS, called M3, is known to cause extremely invasive infections leading to an unusually high degree of severe illness and death.

*Advance:* In a recent study, NIH researchers and their colleagues sought to understand why some GAS strains cause severe infections while others lead to milder illnesses. To accomplish that goal, they studied the GAS genome. By comparing the complete genetic blueprints of bacterial strains from people with different GAS infections, the researchers identified specific genes that may be linked to disease severity. In this case, the researchers determined the complete genetic blueprints of an M3 GAS strain isolated from a person with toxic shock syndrome. When they looked closely at the unique regions of the M3 genome, they found telltale genetic markers indicating that bacteriophages had brought in many of the M3 genes. Analysis of these genes and the proteins they encode indicated that the viruses had imported new genes to create new, virulent GAS strains. Among the unique genes, the researchers identified several that produce bacterial toxins and enzymes that may contribute to the highly infective nature of M3 GAS bacteria, including one that resembles an enzyme in snake venoms.

*Implications:* This discovery highlights an important mechanism for bacterial evolution and explains how new virulence genes are acquired. The toxins and enzymes produced by these genes represent potential targets for vaccines, diagnostics, and new drugs to prevent or treat severe infections. The results of this study also suggest that the exchange of genetic material mediated by bacteriophages may play a critical role in the emergence of new and virulent strains of GAS bacteria. The study also shows how data from bacterial genome sequencing efforts can be used to gain new insights about infectious diseases.

Beres SB, Sylva GL, Barbian KD, Lei B, Hoff JS, Mammarella ND, Liu M-Y, Smoot JC, Porcella SF, Parkins LD, McCormick JK, Leung DYM, Schlievert PM, Musser JM: Genome sequence of a serotype M3 strain of group A streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. Proc Nat Acad Sci 99: 10078-10083, 2002.

## Inflammation Control is Mediated by Adenosine Level

*Background:* One of the most perplexing questions in the study of the immune system is how the body limits inflammation – the tissue swelling that is accompanied by pain, redness, and heat. Inflammation is the body's protective response to tissue damage, injury, or invasion by infectious microbes. It is a complex response orchestrated by various components of the immune system and is usually "turned off" after the damage or infection has been controlled. When inflammation is left unchecked, it can damage tissue and contribute to a host of diseases, such as asthma, rheumatoid arthritis, and chronic hepatitis. Researchers knew that when tissue damage from prolonged inflammation becomes excessive, oxygen levels in the damaged area fall, leading to increased levels of a compound called adenosine outside the cells. Adenosine plays many important roles in the body, but its function in the immune system had not been fully explored.

*Advance:* After a decade of research on this question, investigators at NIH have found that the body may control inflammation through cell surface molecules that can sense runaway inflammation and tissue damage and signal the body to halt the inflammatory response. NIH researchers theorized that excess adenosine acts as a signal of too much inflammation and tissue damage, and that when adenosine attaches to specialized molecules on the surface of cells, known as adenosine receptors, it initiates a chain reaction that slows and eventually stops inflammation. The researchers tested this theory in genetically engineered mice that lacked the adenosine receptor but were identical to normal mice in every other way. Injections of even low doses of substances that cause inflammation led to extensive tissue damage and even deaths in mice that did not have adenosine receptors, but caused little or no damage in normal mice. Further tests showed that no other type of receptor could fully compensate for the lack of adenosine receptors. Mice without this critical molecular brake cannot halt inflammation.

*Implications:* The discovery that adenosine receptors play a crucial role in limiting inflammation and tissue damage has many implications for therapy. Drugs that target adenosine present outside of cells or adenosine receptors on the cell surface might offer effective new treatments for controlling excessive inflammation in a wide range of diseases. The finding may also allow researchers to develop strategies for enhancing the inflammatory response as a way to make better vaccines and antitumor drugs.

Ohta A, Sitkovsky M: Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature 414: 916-920, 2001.

## Older People Achieve Their Goal of Dying at Home

*Background*: National surveys have shown that many elderly and terminally ill people in America would prefer to die at home or in a hospice. Common reasons for avoiding institutional care at the end of life include the fear of painful, invasive, impersonal, and highly technological treatments to extend life, and the desire to maintain personal choice and dignity. However, over half of the deaths among this population still occur within the hospital setting. The Program for All-inclusive Care for the Elderly (PACE), a community-based managed care program for Medicare recipients aged 55 and older, integrates primary, acute, and long-term care services to promote continuity and communication between providers and recipients of care, toward the goal of enhancing personal control in care at the end of life.

*Advance:* Researchers examined the records of over 2,000 PACE decedents to determine their place of death. A characteristic of the PACE program is discussion, planning, and implementation of advance directions to document patient preference for care. In the general elderly population, 20 percent die at home and 44 percent die in the hospital. However, among PACE participants those numbers were almost reversed, with 45 percent dying at home and 21 percent in the hospital. Nursing home deaths, which make up most of the remainder, were roughly equal in both groups. Terminally ill PACE participants with a do-not-resuscitate (DNR) order were 7 percent less likely to die in a hospital. In a surprising finding, PACE participants with an informal caretaker in the home were more likely to die in the hospital, possibly because these caretakers, often a spouse or family member, may have difficulty in facing the stress of accepting the death of their loved one.

*Implications:* Dying at home is not the preference for all patients, nor does it always achieve an "ideal" passing. However, honoring the desires of individuals is an important component of quality of care at the end of life. Health care services for the elderly and for those at the end of life are often fragmentary and disjointed. Elders who sign an advance directive may wish to die with minimal intervention, but their written desires may not be honored in the hospital setting, and hospital staff may not be aware of or have immediate access to other patient records regarding care preferences. This study shows that the PACE helps elders both develop an advance directive and maintain continuity of care, thereby increasing the likelihood that their wishes will be followed. The PACE program may also help lower the costs of care for these patients. With its comprehensive focus and emphasis on communication and advanced planning, the PACE appears to improve adherence to participants' wishes for end-of-life care.

Temkin-Greener H, Mukamel DB: Predicting place of death in the Program for All-inclusive Care for the Elderly (PACE): participant versus program characteristics. The Journal of the American Geriatrics Society 50(1): 125-135, 2002.

## Role of Telomeres in Cellular Senescence

*Background:* In culture, human cells have an inborn "counting mechanism" that tells them when to senesce, or stop dividing. Each time a cell replicates, the ends of each chromosome, called telomeres, get shorter, and once the telomeres get too short, they trigger a "senescence program" that arrests the cell's growth. Loss of telomere function can lead to genetic instability. However, mouse cells also senesce in culture, in spite of having telomeres that are extremely long compared to human cells, suggesting that the telomeres' length *per se* is not what causes cells to stop dividing. Researchers are investigating the mechanisms through which telomeres trigger cellular senescence.

*Advance:* In a recent study, investigators induced overactivity of the protein TRF2, which binds to telomeres and protects chromosomes from damage, in normal human cells. They found that while TRF2 increased the rate of telomere shortening, it did so without accelerating senescence. The results suggest that the senescence program is triggered by changes in the "protection state" of critically shortened telomeres, rather than their length – in other words, the cell detects the likelihood that a shortened telomere will lead to genomic instability, regardless of the length of the telomere itself. In a related study, investigators crossbreeding mice with short and long telomeres observed that in these mice, the senescence program was triggered by the shortest telomere, rather than the average telomere length. This finding suggests that the shortest telomeres in a cell become unstable and unleash the senescence program in order to avoid the propagation of genetically unstable cells.

*Implications:* These data extend previous findings regarding telomere length and aging, and allow a deeper insight into the conditions under which cells senesce. The results also indicate that average telomere length, the parameter most commonly measured by most investigators in the field, may not be biologically relevant.

Karlseder J, Smogorzewska A, de Lange, T: Senescence induced by altered telomere state, not telomere loss. Science 295: 2446-2449, 2002.

Hemann MT, Strong MA, Hao LY, Greider, CW: The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 107: 67-77, 2001.

## A New Mouse Model of Accelerated Aging Provides Insights Into the Aging Process

*Background*: Scientists hypothesize that damage to DNA that occurs during normal metabolism may contribute to the aging process, although the mechanisms remain poorly understood. Incomplete repair of such damage, according to the theory, leads to its accumulation over time and may lead to cellular dysfunction. Studying animal models such as mice that have defects in DNA repair could provide clues to this and other contributors to aging processes in humans.

*Advance*: NIH-supported investigators recently created a transgenic mouse carrying a mutation in the *Xpd* gene, which codes for an enzyme involved in both repair of DNA damage and transcription of DNA into RNA (an important first step in gene activation). The activity of this enzyme is significantly reduced, but not totally absent, in these mice, who have substantially impaired transcription and mildly impaired DNA repair. As a result, the mice appear normal at birth (although they are 10 to 20 percent smaller than normal mice), but prematurely develop several signs of aging, including osteoporosis, female infertility, and graying of hair. Their average life expectancy is about half that of normal mice. The investigators suggest that the failure to adequately repair DNA damage in these mice and allow transcription to proceed past the damaged site may trigger programmed cell death, resulting in accelerated functional decline and depletion of cell renewal capacity.

*Implications*: This research strongly supports the hypothesis that DNA damage is implicated in the aging process, especially if it triggers premature cell death. While not an exact model of premature aging, the new mouse model will be useful for studying a number of aspects of aging, including the roles of DNA damage and cell death, as well as the mechanisms through which the genome maintains itself and how such maintenance contributes to longevity.

De Boer J, Andressoo JO, de Wit J, Huijmans J, Beems RB, van Steeg H, Weeda G, van der Horst GTJ, van Leeuwen W, Themmen APN, Meradji M, Hoeijmakers JHJ: Premature aging in mice deficient in DNA repair and transcription. Science 296: 1276-1279, 2002.

Hasty P, Vijg J: Genomic priorities in aging. Science 296: 1250-1251, 2002.

## A Tale of Two Proteins

*Background:* Parkinson's disease (PD), the second most prevalent neurodegenerative disease after Alzheimer's disease (AD), is associated with a progressive degeneration of neurons in an area of the brain known as the substantia nigra. Another hallmark of PD is the presence in the nigra of small, destructive protein deposits called Lewy bodies, which are also present in the brains of AD patients. The protein alpha-synuclein is a major component of these deposits, and mutations in the gene for alpha-synuclein cause a heritable form of PD. A different protein, Hsp70, reduces the toxicity of a similar type of deposit in a Huntington's disease model. Hsp70 is a chaperone protein (it aids in the proper folding of other proteins), and scientists are currently determining whether Hsp70 and/or other chaperone proteins can reduce toxicity of Lewy bodies.

*Advance:* Investigators engineered a fruit fly model that carried genes for human Hsp70 and alpha-synuclein. They found that in the flies, when alpha-synuclein alone was expressed, numbers of neurons decreased by half; when both proteins were expressed, cell numbers did not differ from those in control animals, suggesting that Hsp70 protected the cells from damage induced by the alpha-synuclein. When Hsp70 was mutated to interfere with its function, synuclein-induced damage accelerated; neuron loss induced by synuclein alone occurred within twenty days while the same amount of neuron loss from animals in which the inactive form of Hsp70 was made occurred within a single day.

*Implications:* Chaperone proteins appear to have a protective role in this model of PD. Finding ways to enhance and appropriately target their activity may be an effective approach to treating a number of neurodegenerative diseases that are accompanied by altered protein conformation and aggregation.

Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM: Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. Science 295: 865-868, 2002.

## Prions, Misshapen Proteins, and Out-Of-Shape Brains

*Background:* Prions are infectious proteins that transform a normal cellular protein ($PrP^{C}$) into an abnormal virulent form ($PrP^{Sc}$) that accumulates in the central nervous system, producing fatal neurological disease characterized by sponge-like holes in the brain that result in movement, emotional, sleep, and cognitive disturbances. Some prion diseases include Creutzfeldt-Jakob disease (CJD), Gertsmann-Sträussler-Scheinker disease (GSS), and a variant of CJD (vCJD) associated with bovine spongiform encephalopathy (BSE or Mad-Cow disease). These and other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington disease, now are thought to be diseases of protein conformation in which a misfolded version of a normal cellular protein aggregates and causes disease.

*Advances:* Investigators have made a number of advances in our understanding of prion diseases. In the first study, researchers noted that, although chemically the same, $PrP^{C}$ and $PrP^{Sc}$ differ in structure. A fragment of the mouse prion protein with a single alteration that causes GSS disease can induce this disease in transgenic mice only if it is in the pathological form, indicating that prion proteins must exist in a particular structure to become infectious and produce neurodegeneration. The specificity of the prion structure may also limit transmission of prion diseases between different species. Researchers have demonstrated that breaching of the species barrier involves the generation of prions with different structural templates that slowly accumulate over multiple transmissions in recipients. In another study, investigators identified a neurodegenerative disorder that mimics the symptoms of Huntington disease (HD), but lacks HD's characteristic genetic mutation. Instead, the disorder is associated with mutations in the prion protein gene. Finally, researchers have found that, in mice, specially engineered antibodies can inhibit interaction between $PrP^{C}$ and $PrP^{Sc,}$ which is necessary to $PrP^{Sc}$ replication. In cells treated with the most potent antibody, prion replication was halted and existing prions were rapidly cleared, suggesting that the antibody may cure established infection.

*Implications:* Neurodegenerative diseases of aging are commonly associated with alterations in the structures of normal cellular proteins. The causes for these changes are largely unknown. Studies of prion diseases offer opportunities to understand the mechanisms underlying such changes and their pathological manifestations, and may lead to new preventives and treatments.

Laws DD, Bitter HM, Liu K, et al.: Solid-state NMR studies of the secondary structure of a mutant prion protein fragment of 55 residues that induces neurodegeneration. Proc Natl Acad Sci 98(20): 11686-11690, 2001.

Moore RC, Xiang F, Monaghan J, et al.: Huntington disease phenocopy is a familial prion disease. Am J Hum Genet 69: 1385-1388, 2001.

Peretz D, Williamson RA, Kaneko K, et al.: Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. Nature 412: 739-743, 2001.

Peretz D, Williamson RA, Legname G, Matsunaga Y, Vergara J, Burton DR, DeArmond SJ, Prusiner SB, Scott MR: A change in the conformation of prions accompanies the emergence of a new prion strain. Neuron 34: 921-932, 2002.

## Adult Neural Stem Cells Make Functional Neurons

*Background:* Contrary to widely accepted belief, the adult nervous system can generate new neurons, a process called neurogenesis, from neural stem cells that are found in specific brain regions. Adult neurogenesis occurs in the hippocampus, a brain region important for learning and memory, which shows degenerative changes in aging and Alzheimer's disease. New neurons generated in the adult brain or from neural stem cells grown in culture are most often identified using cell-specific markers. Although the new cells resemble mature neurons, it is unclear whether the new neurons are functional or integrate into existing neural circuits.

*Advance:* Two studies now show that neural stem cells in the adult hippocampus develop essential properties of functional neurons. In the first study, investigators labeled stem cells in the hippocampus of adult mice by tagging them with a protein called GFP. When the hippocampus was examined 2 days after the injection, the GFP-labeled cells looked like immature neurons, whereas by one month the GFP-labeled cells looked and behaved like authentic hippocampal granule neurons. Close examination showed that the new neurons had properties similar to their mature neighbors, and that they received input from other cells. In the second study, researchers isolated stem cells from the hippocampus of adult rat brain and then tagged the cells with the GFP protein. When these tagged stem cells were cultured along with normal hippocampal neurons or astrocytes, support cells that foster neuron growth, they formed neurons with axons and dendrites, which are structures critical for communication with other cells. In fact, these stem cell-derived neurons made functional connections, called synapses, with normal hippocampal neurons and with each other, and released neurotransmitters, the chemical mediators of neuronal communication.

*Implications:* Neural stem cells derived from the adult brain make functional neurons that can integrate into the circuitry of at least some brain regions. These findings could have important clinical implications. The generation of new functional neurons from neural stem cells, either from those present in the brain or from those transplanted into the brain, could be harnessed to regenerate damaged brain tissue, to replace dying neurons, or to enhance the ability of the brain to respond to age-related impairments.

Song HJ, Stevens CF, Gage FH: Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. Nat Neurosci 5(5): 438-445, 2002.

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH: Functional neurogenesis in the adult hippocampus. Nature 415: 1030-1034, 2002.

## Neurons Know Where We're Going

*Background:* "Optic flow" refers to visual processing that provides cues about the direction of an individual's movement and the three-dimensional structure of the visual environment. Studies in monkeys indicate that the area in the brain that processes optic flow is the medial superior temporal area (MST). In humans, optic flow is impaired in patients with probable Alzheimer's disease (AD) and to a lesser extent in normal aged subjects in comparison to younger subjects. Functionally, impaired optic flow responses are associated with inability to orient and maneuver oneself in the environment, such as when driving a car or finding one's way through corridors of a building.

*Advance:* Investigators recorded neuronal activity in the MST of monkeys during movement on a motorized sled. The movement was in a circular path, either clockwise or counterclockwise, in front of a stationary array of small white lights. Gaze was fixated straight ahead. Meanwhile, microelectrodes recorded electrical discharges, a measure of neuronal activity, from the monkey's MST neurons. They found that the activity of many neurons in the MST increased depending on the direction in which the monkey was moving, regardless of its path or location in the room. However, in some cases, neuronal activity also depended on whether the path was clockwise or counterclockwise. Finally, some neurons responded only when the animal was at a certain place in the room.

*Implications:* The investigators concluded that neurons in the MST appear to encode information about direction of heading, path and place. These functions allow an individual to orient spatially in the environment. Since anatomical pathways from MST are associated with other brain areas that connect to the hippocampus (which is involved in AD pathogenesis), MST could play an important role in the disorientation that is seen in AD and other neurodegenerative disorders.

Froehler MT, Duffy CJ: Cortical neurons encoding path and place: Where you go is where you are. Science 295: 2462-2465, 2002.

## Nitric Oxide Controls the Strength of the Heart Beat

*Background*: The heart is the body's most powerful muscle; its fibers stretch and contract to form the heartbeat. During periods of stress, including physical exercise, blood is pumped more rapidly throughout the body, and heart muscle stretch increases in response. Stretch also affects contraction strength; when heart muscle fibers stretch, calcium ions, which regulate contraction, are released from a part of the fiber called the sarcoplasmic reticuluum (SR). The efficiency of this process is critical to the quality of life during periods of good health, as well as during periods of disease.

*Advance*: NIH researchers have identified a significant mechanism that contributes to the heart's strength of contraction during sustained periods of circulatory stress, such as physical exercise. They found that heart muscle stretch activates a particular pathway that generates nitric oxide (NO). NO, in turn, enhances the fibers' capacity to release calcium ions from the SR. When the stretch is increased, as in periods of physical exertion, NO release is increased, strengthening the contraction.

*Implications*: This mechanism could determine an important part of intrinsic cardiac reserve capacity. In addition, the researchers hypothesize that the loss of naturally occurring NO mechanisms in the body could contribute to the development of functional impairments of heart muscle when other compensatory mechanisms fail.

Petroff MG, Kim SH, Pepe S, Dessy C, Marban E, Balligand JL, Sollott SJ: Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+ release in cardiomyocytes. Nature Cell Biology 3(10): 867-873, 2001.

## Diabetes, ApoE_4 and the Risk for Alzheimer's Disease

*Background*: Type 2 diabetes is one of the most common metabolic disorders, and its prevalence increases with age. Complications of diabetes affect several organs, including the brain. For example, diabetes is associated with hardening of arteries inside the brain and leads to vascular changes that cause a decrease in cerebral blood flow. There is also some evidence that diabetes may increase the risk for Alzheimer's disease (AD), the most common form of dementia in the elderly. This risk may be modified by the major AD susceptibility gene, APOE_4.

*Advance*: Researchers evaluated the links between diabetes, dementia, and the APOE_4 allele in a large group of Japanese-American men. They found that participants with both type 2 diabetes and the APOE_4 allele had a risk for AD 5.5 times higher than those with neither risk factor. At autopsy, participants with type 2 diabetes and the _4 allele had a higher number of AD's characteristic neuritic plaques and neurofibrillary tangles in the hippocampus, the region of the brain where AD is thought to start. They also had a higher incidence of amyloid deposition in the blood vessels in the brain.

*Implications*: These results suggest that a very prevalent condition, type 2 diabetes, may be associated with an increased risk for Alzheimer's disease. This risk appears to be heightened for those persons with both type 2 diabetes and a genetic susceptibility related to the APOE_4 gene. Further investigation is needed into the underlying pathology and effects of treatment of diabetes on the incidence of Alzheimer's disease.

Peila R, Rodriguez BL, Launer LJ: Type 2 Diabetes, APOE gene, and the risk for dementia and related pathologies. The Honolulu-Asia Aging Study. Diabetes 51: 1256-1262, 2002.

## Isolation of Neuron-Restricted Precursor Cells from Human Embryonic Stem Cells

*Background:* Cells in the brain and central nervous system differentiate through a multi-step process. As development progresses, stem cells – cells with a unique capacity to regenerate and give rise to many tissue types – generate a class of cells known as precursors or progenitors, which in turn generate the highly specialized cells of the brain and nervous system. Scientists now have the ability to isolate human embryonic stem (hES) cells, and have found that hES cells proliferate and maintain their pluripotency (ability to give rise to different tissue types) in cell culture. Although ES cells show promise in the treatment of an array of diseases and conditions, the mechanisms that regulate the differentiation of hES cells into the various cell types in the body are largely unknown, and methods for isolating particular types of precursor cells from differentiating hES populations have not been established.

*Advance:* NIH researchers have recently developed a method for inducing hES cells to differentiate into neural progenitor cells and neurons. The newly-derived cells exhibit the appearance and properties of cells ordinarily found in the brain and central nervous system.

*Implications*: These data indicate that hES cells could provide a source for neural progenitor cells and mature neurons for therapeutic and toxicological uses. The ability to identify and isolate different types of progenitor cells from hES cell-derived populations will allow these cell populations to be used for basic research and for cell transplantation to repair tissues damaged by disease or injury.

Carpenter MK, Inokuma MS, Denham J, Mujtaba T, Chiu CP, Rao MS: Enrichment of neurons and neural precursors from human embryonic stem cells. Exp Neurol 172: 383-397, 2001.

## Age Does Not Influence the Response to Resistive Strength Training

*Background*: Loss of muscular strength and muscle mass with age (termed sarcopenia) is associated with the development of disability and frailty in the elderly. Resistive strength training has been an effective intervention against sarcopenia in some circumstances. While older people do respond to such training, it is unknown whether there are differences in that response as compared to younger individuals.

*Advance*: Young (20-30 year old) women and men were compared to older (65-75 year old) women and men, before and after a single legged strength training (in which the untrained leg served as a control for measurement purposes), and again after a 6-month whole body strength training program. In response to the 6 months of strength training, both age groups increased strength, and showed similar increases in muscle mass. In addition, both age groups showed similar increases in resting metabolic rates, which generally decrease with age, contributing to increasing weight gain. Following one-leg strength training, muscle satellite cell activation was found to be similar in both young and old subjects. Skeletal muscle satellite cells are important for muscle regeneration and growth, and it has been suggested that age-related declines in satellite cell activation could impact on the response to strength training.

*Implications*: Strength training has a similar response in both younger and older individuals. The positive effects of training are observed equally in old and young individuals. This intervention can slow the progression of sarcopenia in middle aged and elderly individuals, as well as improve the functional capability of the frail.

Roth SM, Martel GF, Ivey FM, Lemmer JT, Tracy B, Metter EJ, Hurley BF, Rogers MA: Skeletal muscle satellite cell characteristics in young and older men and women after heavy resistance strength training. Journal of Gerontology: Biological Sciences 56A(6): B240-B247, 2001.

Roth SM, Ivey FM, Martel GF, Lemmer JT, Hurlbut DE, Siegel EL, Metter EJ, Fleg JL, Fozard JL, Kostek MC, Wernick DM, Hurley BF: Muscle size responses to strength training in young and older men and women. J Am Geriatr Soc 49(11): 1428-1433, 2001.

Lemmer JT, Ivey FM, Ryan AS, Martel GF, Hurlbut DE, Metter EJ, Fozard JL, Fleg JL, Hurley BF: Effect of strength training on resting metabolic rate and physical activity: age and gender comparisons. Med Sci Sports Exerc 33: 532-541, 2001.

## Uncovering the Secrets of Longevity

*Background:* Determining why some individuals live and thrive into old age while others do not has long been a goal of researchers in the field of aging. Increasingly, researchers are focusing on uncovering biological and genetic mechanisms that might explain the exceptional longevity of some.

*Advance:* Using data from a study of 444 families in which at least one member lived to be 100 or older, researchers found that siblings of centenarians had about half the risk of dying throughout their lives compared with people who did not have a centenarian sibling. The investigators found that brothers of centenarians were at least 17 times more likely to reach the age of 100 themselves and sisters were at least 8 times more likely to live at least a century. These findings are supported by research indicating that excess longevity (the difference between observed and expected length of life) is 15 percent heritable, and that the longevity of both siblings and more distant relatives may be predictive of one's own lifespan. Together, these findings point to strong underlying genetic components of longevity.

*Implications:* It appears that biological bases of longevity may exist, and that the increased odds of survival by the siblings of centenarians indicate that longevity is heritable. More research is needed on the biological and genetic components of longevity.

Kerber RA, O'Brien E, Smith KR, Cawthon RM: Familial excess longevity in Utah genealogies. Journal of Gerontology A Biological Sciences and Medical Sciences. 56A(3):B130-B139, 2001.

Perls TT, Wilmoth J, Levenson R, Drinkwater M, Cohen, Bogan H, Joyce E, Kunkel L, Puca A: Life-Long Sustained Mortality Advantage of Siblings of Centenarians. Proc Nat Acad Sci 99(12): 8442-8447, 2002.

## Regulation of a Critical Cell Cycle Checkpoint by the Breast Cancer Gene *BRCA1*

*Background:* Studies in the mid 1990's identified mutations in the *BRCA1* gene that are the major cause of hereditary breast and ovarian cancer, and also play a role in causing other cancers. Women inheriting these mutations have a significant lifetime risk of developing breast cancer and/or ovarian cancer. This discovery has led to a great deal of work aimed at better understanding the function of the BRCA1 gene.

*Advance:* New work by NIH researchers on the BRCA1 gene has helped establish that it likely plays a role in the development of proteins that govern cell proliferation. When DNA, the genetic material of a cell, suffers damage, the cell normally stops reproducing, and activates a complex of proteins that can find and repair the damaged sections of DNA. This "pause and repair" step prevents the replication of damaged cells. Unless the cell carries out this repair step, the damaged DNA can be propagated to daughter cells. If the DNA mistakes are located in critical places, this can lead to cell growth that causes cancer. Studies indicate that mutations in BRCA1, which normally acts as a quality control, can disrupt the ability of cells to recognize DNA damage and halt division.

According to recent experiments, a protein called ATM activates BRCA1 in this important process. But no one has pinpointed the proteins to which BRCA1 then passes the signal. To find the BRCA1 targets, scientists exposed two types of cultured cells – breast cancer cells that naturally lack BRCA1, and the same cells engineered to produce it – to damaging radiation and analyzed their behavior. After the treatment, cells that produced BRCA1 behaved properly. The cells stopped just before they started mitosis, a process in which a cell divides in two. Cells that lacked BRCA1, however, continued to divide.

Two proteins, Chk1 and Chk2, broadcast signals that delay mitosis until damage can be fixed. The researchers wondered whether BRCA1 activates either of these proteins. Both proteins move into action when they are phosphorylated – that is, receive phosphate groups – so the group tested whether Chk1 and Chk2 carried this chemical group. When cells were irradiated, Chk2 became phosphorylated regardless of whether BRCA1 was around. Chk1, on the other hand, received a phosphate only with BRCA1 present, suggesting that BRCA1 was necessary for the change. Further experiments showed that Chk1 also required BRCA1 to add phosphates to its own protein target, supporting the notion that BRCA1 prods Chk1 to do its job. Next, the researchers tested whether BRCA1 and Chk1 contact each other. Antibodies that grab BRCA1 from a mix of cell contents pull out Chk1 as well. Together, the results suggest that BRCA1 normally transmits a signal to the Chk1 protein – a signal that is key if a cell's DNA is damaged.

*Implications:* The study narrows where in the DNA damage pathway BRCA1 functions. But the data do not directly show the link between BRCA1 and the Chk1 protein. Research on the *BRCA1* gene will also lead to greater understanding of the basic mechanisms of many cancers and of cell proliferation. More experiments are needed in order to pinpoint the BRCA1 target.

Yarden RI, et al: BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage. Nat Genet 30: 285-289, 2002.

## Hereditary Form of Prostate Cancer Linked to Gene on Chromosome 1

*Background:* Prostate cancer is one of the most common cancers in American men – an estimated 189,000 new cases occurred in 2001. Family history is the strongest risk factor. The NIH is working diligently to understand the hereditary factors – the genes – that contribute to risk for developing prostate cancer.

*Advance:* This line of inquiry promises to unlock secrets of prostate cancer; secrets whose opening will lead to new methods to both prevent and treat prostate cancer much more effectively. For instance, one current study supported by the NIH suggests that while most men inherit two normal copies of a gene called "RNASEL"– one from their mother and one from their father – some men with hereditary prostate cancer inherit a defective copy of this gene from one of their parents. The normal RNASEL gene makes a specific enzyme that prostate cells need to function normally. While a prostate cell with one normal and one abnormal copy of the gene makes less of this enzyme, it still produces enough to allow the cell to be healthy. However, in such cells, the normal copy of the gene occasionally happens to mutate – to change – so that it, too, is abnormal. If this happens, the cell, and all the prostate cells that derive from it later, produce so little, if any, of this necessary enzyme, that the cell and its progeny are not healthy – indeed, in a manner that we do not yet understand, they grow into a cancer.

Although the RNASEL gene seems to be strongly implicated in some cases of hereditary prostate cancer, much more work needs to be done to determine how commonly it causes or modifies the clinical course of the disease. A larger number of men - both with and without strong family histories, and with and without prostate cancer - need to be studied to see how often mutations in the gene are associated with the disease and how often they occur in men without the disease. To help enlarge the pool of families with this hereditary condition, and to focus on an important high-risk group (African-Americans, where the incidence is almost twice as high as in Caucasians), NIH has established a collaboration with Howard University in Washington, D.C., called the African American Hereditary Prostate Cancer Project.

*Implications:* As these NIH-supported scientists search for genes that cause prostate cancer, they hope to develop better tests to predict or detect the disease, and to develop better treatments based on an understanding of the molecular causes of disease. There is strong evidence that understanding the mechanism of a disorder – for instance, how a mutation in RNASEL changes the working of the gene – will speed up the development of more effective treatments, and ultimately, cures and effective preventive strategies.

Carpten J, et al: Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet 30: 181-184, 2002.

## Low Vitamin C Levels May Be Linked to Massive Brain Bleeding and Lung Failure in Premature Newborns

*Background:* The only proven human requirement for vitamin C, or ascorbic acid, is to prevent scurvy, a disease characterized by bleeding gums, anemia, skin hemorrhages, and death. The normal recommended daily allowance for vitamin C for women is 75 mg daily, which is increased by 10 mg during pregnancy – totaling a little more than the amount in the average-sized orange. However, the overall vitamin C intake can vary greatly in the general public, ranging anywhere from 20 mg to 10,000 mg per day.

*Advance:* Scientists at the NIH and the division of Neonatology at the University of Pennsylvania School of Medicine and Children's Hospital have discovered a possible link between reduced vitamin C availability during pregnancy and the devastating respiratory failure and massive cerebral bleeding that can occur immediately in a newborn following premature birth. These scientists created a mouse model with a defective *Slc23a1* gene, which encodes the protein that transports vitamin C into cells. They discovered that the mouse model could not deliver vitamin C from the blood to many fetal tissues or get it across the placental border. Therefore, the *Slc23a1*gene-deficient mice had noticeably reduced levels of ascorbic acid in their blood and very low or undetectable levels in their brains and other organs.

What surprised the scientists was the discovery that the vitamin C-deprived mice died within minutes after birth due to massive cerebral hemorrhage and complete respiratory failure when their lungs failed to expand. These severe health problems occurred whether the newborn mice were delivered normally after 21 days gestation, or delivered early by Caesarean section, at 18.5 to 19.5 days, to avoid birth trauma. Also, loss of surfactant protein production could not explain the abnormalities in lung function, since levels of a critical protein, surfactant B, were normal. And intracerebral hemorrhage due to vascular fragility, or a defect in collagen processing from low vitamin C levels, similar to the effects of scurvy, were apparently ruled out, since the mice had developed normally while in the womb, including normal weight gain.

The study demonstrated that mice deprived of vitamin C during pregnancy died almost immediately after birth from bleeding in the brain and respiratory failure. In humans, intracerebral hemorrhage and respiratory failure are frequent causes of serious morbidity and death in premature infants. Since 20 percent of the population consumes less than the recommended dietary allowance for vitamin C intake, the study has important implications for what may happen with partial vitamin C deficiency, as well as for the devastating effect of a total absence of vitamin C.

*Implications:* Now, scientists at NIH are investigating whether there are genetic differences in vitamin C absorption or transport, which could render some pregnant women and their fetuses more susceptible to partial dietary deficiency of the vitamin.

Sotiriou S, et al: Ascorbic acid transporter Slc23a1 is essential for vitamin C transport into the brain and for perinatal survival. Nat Med 8(5): 514-517, 2002.

## Genetic Defect Responsible for Brain Disorder Among Amish Babies

*Background:* Over the past 40 years, 61 babies with a birth defect marked by profoundly small head and brain size have been born to 23 nuclear families in the Old Order Amish community in Lancaster County, Pennsylvania. None of the children has lived beyond the age of 14 months, and most die at four to six month of age.

*Advance:* An international team of researchers, led by the NIH, has discovered the genetic cause for a rare form of microcephaly (MCPHA), a devastating brain disorder that has stricken infants among the Old Order Amish for at least nine generations. In their study, the NIH team found that a defect in the gene causes developing cells to lose their normal ability to transport the building blocks of DNA across the inner membrane walls of the mitochondria, tiny structures that function as the cell's metabolic powerhouses. Researchers believe that without this carrying ability, called mitochondrial deoxynucleotide transport, the cell's mitochondria cannot make DNA properly, causing the brain of the unborn child to develop abnormally. The NIH data also indicate that mitochondrial deoxynucleotide transport may play a crucial role in normal prenatal brain growth.

Microcephaly describes the situation where a baby's head circumference is significantly less than the average newborn's head size of 33 to 38 cm (about 13 to 15 inches). Specifically, the head circumference of a microcephalic infant is three standard deviations or more below normal. Babies born with MCPHA have a particularly severe form of the defect, with a head circumference anywhere from six to 12 standard deviations below the average for a normal newborn. Their skulls are very small and their brains undeveloped and malformed.

In addition to brain and skull abnormalities, babies with MCPHA have elevated levels of the biochemical α-ketoglutarate in their urine, a finding that is directly related to MCPHA but still not clearly understood. Scientists also are investigating the mystery of why the children's other organs, such as the heart and liver, seemingly are not affected, even though during development these organs rely on energy production from mitochondria at a metabolic rate similar to the brain.

*Implications:* This study led to a significant finding for all prenatal brain development, making a tie between energy metabolism and brain development. Scientists will look at how this abnormality ties into other genes that are known to cause microcephaly, figure out how the genes interact with each other and then look for other connections between energy metabolism and brain development.

Rosenberg MJ, et al.: Mutant deoxynucleotide carrier is associated with congenital microcephaly. Nat Genet 32(1): 175-179, 2002.

## Autoimmune Link in Juvenile Batten Disease

*Background:* Children who inherit juvenile Batten disease develop normally at first, but symptoms of the disease usually begin to appear between ages 5 and 10. These include vision problems, seizures, behavior changes, slow learning, and clumsiness. As the disease progresses, children suffer increasing from mental impairment, worsening seizures, and progressive loss of sight and motor skills. The disease is usually fatal by the late teens or twenties.

In 1995, scientists identified defects in a gene called CLN3 that cause juvenile Batten disease. The normal function of the gene is not known, and how the gene defects produce the many symptoms of this disorder has remained elusive. Building on the gene discovery, scientists have "knocked out," or inactivated, the CLN3 gene in mice. These knockout mice develop symptoms that resemble the human disorder and allow scientists to more easily study how CLN3 might cause the disease, and to test potential strategies for treatment.

*Advance:* Scientists studying CLN3 knockout mice discovered an immune reaction that disables an important enzyme in the brain. The attack by the body's own immune system inactivates an enzyme called glutamic acid decarboxylase 65 (GAD65). GAD65 normally converts the neurotransmitter glutamate into another neurotransmitter called gamma-aminobutyric acid, or GABA. As a result, the mice had elevated levels of glutamate. Glutamate normally excites nerve cells, increasing their electrical activity. GABA normally inhibits nerve cells, decreasing their electrical activity. Excessive excitation can lead to seizures, or even to nerve cell death through a process called "excitotoxicity." Thus, altered levels of glutamate and GABA may have widespread consequences on the brain. Further studies in the knockout mice also suggested possible indirect effects of these neurotransmitter changes on crucial supporting cells of the brain called astrocytes and on the expression of other genes. Finally, based on the insights from studying knockout mice, the researchers examined children with Batten disease. They found that 20 out of 20 children tested had antibodies to GAD65, suggesting that similar mechanisms come into play in the humans as in the mice.

*Implications:* This is the first study to show that autoimmunity may play a role in a pediatric neurodegenerative disorder of genetic origin. The findings provide a crucial clue to what goes wrong in Batten disease, and may suggest new approaches to treating the disorder. Autoantibodies to GAD have been detected in an adult neurological disorder called stiff person syndrome that causes progressive muscle rigidity and spasms, symptoms that may occur late in Batten disease. Last year, an NIH clinical trial showed that immunotherapy significantly improved symptoms in stiff person syndrome, so this, or other immune targeted approaches might be useful to slow Batten disease.

Chattopadhyay S, et al.: An autoantibody inhibitory to glutamic acid decarboxylase in the neurodegenerative disorder Batten disease. Hum Mol Genet 11(12): 1421-1431, 2002.

## Progress in Understanding the Molecular and Genetic Basis of Inherited Epilepsy

*Background:* In epilepsy, the normal pattern of nerve cell activity in the brain becomes disturbed. This can cause recurrent seizures, sometimes with loss of consciousness, as well as strange sensations, emotions, and behavior. Head trauma, illness, toxic substances, and abnormal development of the brain are among the many causes of epilepsy. Recently, scientists have made substantial progress in identifying genes that, when defective, can cause epilepsy. By studying genes implicated in the inherited forms of epilepsy, scientists can discern crucial clues about how the brain activity goes awry that may apply to all forms of the disease. Gene findings also allow scientists to develop animal models that help in studying the disease and testing treatments. So far, most of the defects implicated in inherited epilepsy are in genes for ion channel proteins. Ion channels regulate how positively and negatively charged ions, such as sodium and potassium, enter and leave cells, determining the electrical activity of nerve cells and the brain as a whole.

*Advance:* Three new findings in the molecular biology and genetics of epilepsy illustrate progress in understanding how gene defects lead to seizures, and how gene findings can provide new clues about what causes this disease. One report focuses on mutations in the sodium ion channel that are associated with a syndrome called generalized epilepsy with febrile seizures plus (GEFS+). The investigators discovered that the mutated channels failed to shut off completely after they were activated, rendering nerve cells susceptible to the kind of repetitive electrical activity that can underlie epilepsy. Two other studies are notable because they identify, in a mouse and in people, gene defects not associated with ion channels that can cause epilepsy. The human study showed that the gene LGI1 is responsible for a syndrome called autosomal-dominant partial epilepsy with auditory features, in which affected persons have recurrent seizures and may "hear" sounds that aren't there. This gene has previously been associated with growth in tumors. It may help control growth and migration of developing nerve cells in the brain. Subtle aberrations in these developmental processes are linked to epilepsy in many cases. The animal study identified a gene defect that causes mice to have seizures in response to sudden loud noises. Here again the gene does not appear to be an ion channel. Although its normal function is not clear, the resemblance of the gene to known genes suggests a few possibilities that may present novel mechanisms for seizure development.

*Implications:* Only a small percentage of epilepsy appears to be inherited via defects in single genes. However, as in other neurological disorders, studying these less common subtypes is providing essential clues toward understanding all forms of epilepsy and identifying potential targets for drugs or other interventions.

Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL Jr: Molecular basis of an inherited epilepsy. Neuron 34: 877-884 2002.

Kalachikov S. et al: Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. Nat Genet 30: 335-341, 2002.

Skradski SL, Clark AM, Jiang H, White HS, Fu Y-H, Ptacek LJ: A novel gene causing a Mendelian audiogenic epilepsy. Neuron 31: 537-544, 2001.

## Understanding How Inherited Defects Cause Facioscapulohumeral Muscular Dystrophy

*Background:* Facioscapulohumeral muscular dystrophy (FSHD) is a degenerative disorder that causes muscle weakness and atrophy. The name reflects the characteristic pattern of weakness, including the face (facio), shoulders (scapulo), and upper arms (humeral), but the severity and progression of this disease are highly variable. About 10 years ago, scientists found that nearly all people with FSHD are missing part of a segment of chromosome 4. This region, called D4Z4, is a Variable Number Tandem Repeat structure (VNTR), meaning that it normally consists of a variable number of repeats of a particular DNA sequence. Most unaffected people have between 11 and 150 of these D4Z4 repeats, but when the number goes below 11, FSHD arises. People with 3 or fewer repeats have severe FSHD that begins in childhood, while those with more copies have a milder form of the disease that begins in the teens or early adulthood. The chromosome defect does not appear to delete or directly disrupt a gene, so how these alterations result in the disease has been a mystery for a decade since the deletion was first discovered.

*Advance:* Scientists have discovered that the chromosomal alterations in FSHD lead to over-expression (activity) of other genes which are on chromosome 4, but outside of the D4Z4 region. It is likely that the inappropriate activity of one or more of these genes causes the disease. The VNTRs (repeats) normally help regulate other genes through a process called repression, by which complexes of regulatory proteins bind to these regions of DNA and prevent nearby genes from being read out to make proteins. Several genes are affected, and their over-activity appears to be specific to muscle tissue. Researchers are attempting to determine which gene (or genes) might be responsible for muscle degeneration. A good candidate is a gene called ANT1 which helps regulate a process called apoptosis, or "cell suicide," that is involved in muscle degeneration. The findings also explain the autosomal dominant pattern of inheritance, through which a person can inherit FSHD from either parent, as well as accounting for the tendency for people with the fewest repeats to have the most severe disease.

*Implications:* These findings present a major advance in understanding a common form of muscular dystrophy. The absence of a direct effect on a gene has baffled scientists since the underlying chromosomal defect was discovered a decade ago. Pursing this lead to determine exactly which gene(s), through over-expression, causes muscle to degenerate is an obvious avenue for further research that may lead to strategies for treatment. More generally, the results may provide insights into the function of DNA repetitive elements, such as VNTRs, and their potential role in other human diseases. This is the first time such a mechanism has been implicated in human disease. There are other VNTRs in chromosomal regions that are potentially associated, for example, with type 1 insulin dependent diabetes mellitus and with bladder cancer, so it is possible to speculate that these repetitive elements may have a role in regulating gene expression similar to that of D4Z4.

Gabellini D, Green MR, Tupler R: Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. Cell 110: 339-348, 2002.

## Minocycline Delays Onset and Slows Progression of ALS in Mice

*Background:* ALS, or Lou Gehrig's disease, progressively attacks nerve cells that control movement, leading to paralysis and death. Riluzole, the only drug approved to treat the disease, slows the time course by only a few months. Approximately 90 percent of patients still die within five years after symptoms begin. Although most cases of ALS are not inherited, several years ago scientists identified the gene defects responsible for a less common, inherited form of ALS. Using genetic engineering, researchers developed strains of mice that express this gene and mimic the human disease. The ALS mice allow scientists to study the underlying biology of ALS and to test potential treatments.

Studies in ALS mice implicated a process called apoptosis in the disease. During apoptosis, or "cell suicide," cells activate a program of biochemical steps that results in their death. Apoptosis is a normal process during development and in adults that efficiently eliminates cells that are no longer needed, but the process also comes into play in many neurological disorders. Scientists studying apoptosis in cell culture and in other animal models of neurological disorders have noted that the drug minocycline, which has been approved by the FDA as an antibiotic, somehow interferes with apoptosis.

*Advance:* Researchers have now shown that injections of minocycline slow the onset and delay progression of symptoms in ALS mice. The effects are similar in magnitude to those of riluzole. The research team went on to examine at which steps of apoptosis the drug acts, relying upon descriptions of the cell death process that had been worked out through years of basic research studies by many laboratories. Minocycline interferes with an early step in apoptosis, by preventing the release of the enzyme cytochrome c from mitochondria, a compartment of cells that is involved with extracting energy from food.

*Implications:* Because minocycline works differently from riluzole, a combination may be more effective than either drug alone. Researchers are investigating that possibility. Designing drugs that more specifically attack this early step in the cell death cascade may also be a useful strategy for ALS and for other diseases in which apoptosis comes into play. More generally, the findings illustrate the importance of animal models of human diseases and of studying rare inherited forms of disease, which can yield animal models as well as insights about the disease process. The study also demonstrates how recognition of common underlying disease processes, such as apoptosis, help bring results from one disorder to bear on others. The effectiveness of minocycline supports the strategy of testing against neurological disorders drugs that have been already approved by the FDA for other purposes. This year NINDS initiated a systematic program to do that. The Institute supported researchers to test a set of 1040 FDA approved drugs in 29 simple *in vitro* tests related to neurological disorders. The scientists met in April 2002 and are proceeding to share data and identify the best candidates for further studies in mouse models of disease.

Zhu S, et al: Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 417: 74-78, 2002.

## Delivering Therapy to the Brain with Molecular Trojan Horses

*Background:* The blood-brain barrier (BBB) excludes most chemicals in the general blood circulation from entering the brain. Although this property normally helps protect the brain from potentially toxic substances, it also prevents most potentially therapeutic drugs from entering the brain. Only small molecules that are lipid (fat) soluble can enter, which excludes an estimated 98 percent of potential drugs. The cells of brain capillaries (small blood vessels) that make up the BBB contain specific transport systems , which latch onto the molecules that the brain needs from the general circulation and carry those specific chemicals into the brain, circumventing the BBB.

*Advance:* Scientists have developed a "Trojan horse" strategy to deliver drugs across the BBB. The basic idea is to trick one of the specific transport systems into carrying a drug into the brain, much as the legendary Greeks hid inside the Trojan horse, which the Trojans carried across the walls of Troy. In one demonstration of the approach, researchers linked a potentially therapeutic molecule called basic fibroblast growth factor (bFGF) to an antibody, which attached to the transferin transporter. The tranferin transporter brings iron across the BBB bound to the iron carrier protein transferin. Basic FGF is one of a large group of natural nerve cell growth and survival factors, or neurotrophic factors, that have enormous potential for treating many neurological disease, but are excluded by the BBB. When rats were intravenously given the transportable version of bFGF an hour after an experimental stroke, the area of damaged brain was reduced by 70 percent. The normal form of bFGF, which cannot get into the brain, had no such effect.

*Implications:* This strategy for overcoming the BBB by engaging the transport systems can potentially be used for a wide variety of drugs in treating many neurological disorders. In another set of experiments, for example, researchers used this strategy to treat brain tumors in mice with a gene therapy agent, extending survival by 100 percent. These studies highlight the importance of gaining a better understanding of the BBB and its transport systems, as emphasized by the recent NIH Brain Tumor Progress Review Group and the Stroke Progress Review Group, as well as strategic planning panels.

Song B-W, Vinters HV, Wu D, Pardridge WM: Enhanced neuroprotective effects of basic fibroblast growth factor in regional brain ischemia after conjugation to a blood-brain barrier delivery vector. Journal of Pharmacology and Experimental Therapeutics 301(2): 605-610, 2002.

Zhang Y, Zhu C, Pardridge WM: Antisense therapy of brain cancer with an artificial virus gene delivery system. Molecular Therapy 6(1): 67-72, 2002.

Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nature Reviews Drug Discovery 11:131-139, 2002.

## Watching Nerve Cells and Molecules in Living Animals

*Background:* The nervous system undergoes remarkable changes during development, and even in adults. Nerve cells arise from precursors, migrate widely in the brain, and sometimes die as a normal consequence of how the brain develops. Nerve fibers, both axons and dendrites, grow and retract. New synapses form and old ones may change in strength. Until recently, scientists have been able to study these dynamic processes only indirectly. The usual approach is to examine cells or tissue from different animals at various time points, for example animals at different ages. However, deducing the patterns of change from such studies, much less the governing rules, is seriously hampered. It is often impossible to understand changes at the level of single cells and nerve fibers at which critical brain changes are occurring.

*Advance:* Over the last several years, innovations in microscopy, new cell and tissue dyes, and genetic engineering of markers have dramatically increased the ability of researchers to study dynamic processes in the nervous system at the cellular and even the molecular level in living animals. Two recent examples at the neuromuscular junction illustrate the progress. The neuromuscular junction is the synapse, or functional connection, between skeletal muscles and the nerve cells that activate them. It has served for decades as a good model for understanding the smaller and less accessible synapses of the brain. In one recent investigation, scientists relied upon strains of mice genetically engineered so that different subsets of nerve cells connecting to muscle fluoresced in different colors. The researchers followed the growth and retraction of the nerve fibers using a technique called confocal microscopy, which allowed them to resolve fine structures even below the surface of tissue. The scientists could observe and make 3-dimensional reconstructions over time as the intermingled early nerve connections from different cells matured into the more orderly adult pattern. Similarly, in other experiments at the neuromuscular junction, scientists were able to label individual receptors, the molecules by which cells respond to neurotransmitters. Following the labeled receptors revealed a remarkable dynamism that underlies the functional stability of the neuromuscular connection.

*Implications:* Various technical advances have come together to allow scientists to follow key events in the nervous system, at the level of cells and molecules, in their natural setting, the living animal. Throughout the history of neuroscience, insights about development have been a key for understanding the nervous system in health and disease, and for developing innovative therapeutic strategies, such as stem cell transplantation, gene therapy, behavioral interventions, and the use of natural nerve cell growth and survival molecules. The more recent recognition of the extent to which even the adult brain changes heightens the need for techniques to monitor and understand the dynamic anatomical features of the nervous system in health and disease.

Lichtman JW, Fraser SE: The neuronal naturalist: watching neurons in their native habitat. Nat Neurosc Suppl 4: 1215-20, 2001.

Keller-Peck CR, Walsh MK, Gan W-B, Feng G, Sanes JR, Lichtman JW. Asynchronous synapse elimination in neonatal motor units: studies using GFP transgenic mice. Neuron 31: 381-394, 2001.

Akaaboune M, Grady RM, Turney S, Sanes JR, Lichtman JW. Neurotransmitter receptor dynamics studied in vivo by reversible photo-unbinding of fluorescent ligands. Neuron 34: 865-876, 2002.

## Animal Studies Yield Progress Toward Repairing Injured Spinal Cord

*Background:* The spinal cord, like the brain, shows very limited self-repair following injury, leading to a lifetime of disability for more than two hundred thousand people in the U.S. alone. Over the last several years, scientists have determined that the lack of functional regrowth reflects a combination of factors. These include physical barriers (scar tissue), chemical growth inhibitors that are intrinsic to the adult spinal cord or arise following injury, and a lack of growth ability, or "growth ready" state, in nerve cells themselves. Researchers have begun to devise strategies in animal models to overcome each of these obstacles to repairing the injured spinal cord.

*Advance:* Several recent reports illustrate how researchers are focusing on the entire range of obstacles to spinal cord repair, with several strategies promoting partial recovery in animal models. Recent experiments addressed the lack of intrinsic growth ability of adult nerve cells, demonstrating that a regulatory chemical called cyclic AMP could shift nerve cells to a growth state and short-circuit messages from external growth inhibitors. Other investigators have designed a drug to help growing nerve fibers overcome the growth blocking influence of a chemical called *nogo,* which is present on the surface of glial (supporting) cells in the spinal cord. Research teams have also heeded the physical and chemical changes in the spinal cord that provide barriers to growth, such as glial scarring. One group of investigators found that a combination of neurotrophic factors (natural growth and survival chemicals) and transplanted fetal tissue could encourage growth, with best results occurring if the treatment was given after a delay to allow the injured spinal cord tissue to stabilize. Another recent study helped overcome barriers to growth with a multicomponent polymer scaffold seeded with neural stem cells. Results from European laboratories, building on earlier efforts in the U.S., have also shown encouraging success using enzyme treatment to remove a chemical called chondroitin sulfate that blocks growth of nerve fibers.

*Implications:* No single strategy for spinal cord repair, used alone, is likely to be successful. Most scientists now believe that a combination of treatments designed to overcome the multiple obstacles to spinal cord repair will someday help individuals with spinal cord injury to regain many lost functions. These examples illustrate only a few of the several avenues for repairing the spinal cord that scientists are actively investigating. Practical and safety concerns must be addressed before these therapies can be tested in human patients. For example, some of the drugs used in these studies are too toxic for human use, and uncontrolled growth or inappropriate nerve cell connections could do more harm than good. However, a cautious optimism is emerging that spinal cord repair is an achievable goal.

Neurmann S, Bradke F, Tessier-Lavigne M, Basbaum AI: Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. Neuron 34: 885-893, 2002.

Qiu j, Cai D, Dai H, McAtee M, Hoffman PN, Bregman BS, Filbin MT: Spinal axon regeneration induced by elevation of cyclic AMP. Neuron 34: 895-903, 2002.

GrandPre T, Li S, Strittmatter SM: Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature 417:547-551, 2002.

Coumans JV, Lin T-S, Dai HN, MacArthur L, McAtee M, Nash C, and Bregman BS. Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. J Neurosci 21(23): 9334-9344, 2001.

Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, Langer R, Snyder EY: Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc Nat Acad Sci 99(5): 3024-3029, 2002.

## Genetic Analysis of Childhood Brain Tumors Improves Diagnosis, Predicts Survival, and Provides Insights Toward Developing More Effective Therapies

*Background:* Brain tumors are second only to the leukemias as a cause of cancer deaths in children. Even children with tumors that respond to treatment suffer side effects that can include learning disabilities and other lasting problems. The difficulties of confronting brain tumors are compounded by the large number of different types – more than 100 – with different prognoses and responses to therapy. Diagnosis of tumors is normally based on clinical and pathological factors, such as whether a tumor has spread, and the appearance of the tumor cells under a microscope. To improve diagnosis, scientists are capitalizing on the recognition that cancer is a genetic disease – that is, tumors arise when damage to a succession of genes, taken together, releases cells from the normal controls on growth.

*Advance:* New studies show that "gene fingerprints" can help classify types of childhood brain tumors, improve diagnosis, and provide insights toward understanding the causes and developing treatments. In one study, researchers focused on a class of tumors called medulloblastomas. Although medulloblastomas are the most common malignant brain tumors of childhood, their relationship to other types of tumors is not clear, and patients' response to therapy is highly variable and difficult to predict. The team used microarrays or "gene chips" to monitor the activity of thousands of genes in samples taken from tumors. The gene analysis allowed researchers to distinguish medulloblastomas from other types of tumors, and to accurately predict the clinical outcome in children with these tumors. The results provided important information about how medulloblastomas are related to other types of brain tumors and implicated several genes not previously recognized as playing a role in medulloblastomas that may reveal additional targets for drugs. Another study of childhood brain tumors focused on the role of the gene *p53* in children with malignant gliomas, a type of tumor with a very poor prognosis. High activity of *p53* was strongly associated with a poor outcome, as has been observed in several types of cancers in adults.

*Implications:* Genetic analysis may help to improve treatment for children with brain tumors. Doctors may be able to identify patients at relatively low risk and use less aggressive therapy, minimizing long term side effects such as learning disabilities. The genetic clues are also providing avenues for developing new therapies.

Pomeroy SL, et al: Prediction of central nervous system embryonal tumour outcome based on gene expression. Nature 415: 436-442, 2002.

Pollack IF, et al: Expression of p53 and prognosis in children with malignant gliomas. New Engl J Med 346(6): 420-427, 2002.

Berman DM, et al: Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 297:1559-1561, 2002.

## Microarray Analysis Yields New Targets for Drugs in Multiple Sclerosis

*Background:* Multiple sclerosis is a serious neurological disorder that often attacks young adults. Symptoms may include not only progressive paralysis and problems with coordination and balance, but also visual deficits, a variety of other sensory disturbances, and cognitive impairment. Patients may suffer a chronic course or suffer recurrent relapses, although the disease progressively worsens in most people. The diversity of symptoms reflects damage to nerve fibers in various regions of the white matter in the brain and spinal cord, and particularly to the insulating myelin covering that gives white matter its appearance. The lesions (damage) result from attack by cells from a person's own immune system. Current drugs partly ameliorate symptoms and somewhat slow the course of disease by modulating this immune response. However, the nature of the immune reactions are not well understood and treatments are far from adequate.

*Advance:* Using microarray analysis, a team of researchers has advanced our understanding of what goes wrong in multiple sclerosis, and has identified new targets for drugs to combat the disease. Microarrays allow scientists to study the activity of thousands of genes at once, giving an unprecedented window on cellular functions. Researchers compared the activity of genes in autopsy samples of acute multiple sclerosis brain lesions, in chronic "silent" lesions without inflammation which produce no overt symptoms, and in normal brain tissue. The activity of several genes differed significantly between the active and silent lesions, as well as between diseased and normal brains, providing new insights into the underlying biology.

Microarray results from two of these genes, both related to immune system function, were chosen for further examination in mice with experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. The Fc receptor is upregulated in silent lesions, and, as predicted, acute EAE was less severe and chronic disease was absent in strains of mice lacking Fc receptors. Also consistent with the microarray results, treatment of mice with granulocyte colony stimulating factor (G-CSF) decreased severity of early stages of EAE but had no effect later.

*Implications:* To a biologist, understanding which genes are active is a key to understanding the behavior of cells in health and in disease. Large scale analysis of patterns of gene activity by microarray analysis yields new insights into multiple sclerosis. The follow-up experiments in the EAE mouse model validate this approach and demonstrate how gene analysis may open new avenues for possible therapies. Similar strategies using microarray analysis are underway for a wide variety of neurological disorders.

Lock C, et al: Gene microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 8(5): 500-508, 2002.

## Cystamine Prolongs Survival and Decreases Abnormal Movements in an Animal Model of Huntington's Disease

*Background:* Huntington's disease is a fatal neurodegenerative disorder. Although the inherited gene defects are present at birth, movement problems, personality disruption, and mental deterioration typically first become apparent in middle age and progressively worsen over several years as more brain cells die. In 1993, after a decade long search, a team of scientists found the gene, which, when defective, causes this disease. The gene carries an abnormal "triplet repeat" – a repetition of a three letter "word" of the genetic code. So, the defective protein produced by the gene, called huntingtin, carries many copies of the protein building block glutamine, which have been coded for by the triplet repeat. Researchers suspect that these extra glutamines somehow damage the brain, but they don't know how. Scientists have genetically engineered mice that have expanded glutamine repeats. These mice develop a progressive, fatal neurological disease that resembles human Huntington's disease, allowing scientists to study in detail how the disease progresses, and to test treatments.

*Advance:* The drug cystamine prolongs survival and decreases abnormal movements in mice with the Huntington's disease gene defect. Cystamine was tested because it blocks the activity of the enzyme transglutaminase, which acts on the extended glutamines, and is thought to play an important role in the progression of Huntington's disease. The ability of injected cystamine to reach the brain and inactivate the enzyme was confirmed by direct experiments. Using "gene chips," which can analyze the activity of thousands of genes at once, the research team went on to identify two genes that had increased activity in mice treated with cystamine, as well as in brain tissue collected post mortem from Huntington's disease patients. The proteins produced by these genes are known to protect brain cells from damage, based on earlier experiments in a fruitfly model of neurodegeneration. So, in addition to its direct effects on transglutaminase, cystamine may enhance a natural attempt by the brain to counteract the effects of disease.

*Implications:* These findings suggest that cystamine, or other drugs that inhibit the transglutaminase enzyme, might be useful for treating Huntington's disease in people. Because cystamine appears to act by a different mechanism than other drugs that have recently shown promise in mouse models of Huntington's disease, a combination of drugs might offer a more effective result. Huntington's disease is one of several neurological diseases in which glutamine repeats have been implicated, so the findings may have implications for treating other diseases as well.

Karpuj MV, et al: Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. Nat Med 8(2): 143-149, 2002.

## Shedding Light on Biological Clocks

*Background*: Anyone who has traveled across time zones or worked at night has dealt with the temporary changes in eating and sleeping habits that usually accompany the adjustment to the new environment. Many living organisms are subject to cyclical variations in mental alertness, sleep-wake patterns, eating habits, and hormonal levels within a given 24 hour cycle. This circadian (daily) clock synchronizes the biological activities of the organism to environmental changes such as light and darkness, temperature, and seasons. Disruption of this clock usually has immediate consequences for the organism. Early experiments had established that this circadian clock might be controlled or reset by light. What has eluded researchers until recently are the cellular events that utilize light to synchronize the circadian clock in a process called photic entrainment. Vision requires light stimulation of specialized sensory neurons called photoreceptors in the retina. Retinal ganglion cells (RGCs) further encode this visual information, transmitting it to either higher visual centers of the brain, such as the optic nerve, or to a non-visual area, the suprachiasmatic nucleus (SCN). The SCN is the circadian rhythm pacemaker of the brain, driving daily biological activities. Experiments aimed at identifying the role of the SCN and the retina in regulating the biological clock found that destruction of the SCN lead to complete disruption of circadian-linked behaviors. Surprisingly however, the SCN could function even in the absence of retinal photoreceptors. Recent research has done much to "shed light" on this quandary, linking the two observations into a new framework for understanding the cellular mechanisms of the circadian clock.

*Advance*: Two important findings were recently reported. First, anatomical and molecular tools have described a protein, melanopsin, that is present in a subset of RGCs in the retina. Melanopsin belongs to a family of proteins, called photopigments or opsins that are found in retinal photoreceptors and are essential for vision. Those RGCs that contain melanopsin project to the SCN, the circadian pacemaker. Second, electrophysiological studies show that RGCs projecting to the SCN respond directly to light stimulation. These responses are identical to those seen during measurements of the intrinsic circadian clock in the SCN. Thus, a subset of light responsive-RGCs that project to the SCN and contain the photopigment melanopsin, directly controls the circadian pacemaker to drive cyclical biological activities.

*Implications*: This work provides scientists with a new framework to understand and ultimately control complex behaviors such as eating and awake activity. Information from these studies may also ultimately help individuals suffering from the temporary but debilitating effects of jet lag or chronic sleep disorders.

Hattar S, Liao H-W, Takao M, Berson DM, Yau K-W: Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. Science 295: 1065-1070, 2002.

Berson DM, Dunn FA, Takao M: Phototransduction by retinal ganglion cells that set the circadian clock. Science 295: 1070-1073, 2002.

## Protein Integrity May Be Key to Lens Transparency

*Background*: Cataract, an opacity of the lens of the eye that interferes with vision, is the leading cause of blindness in developing countries. In the U.S., more than 1.5 million cataract surgical procedures are performed each year, largely to correct age-related cataracts. Age-related cataract formation is believed to result from the complex effects of aging on normal physiological processes. Because the end-result, cataract formation, is in most cases far removed in time from the initial insult, exacting a cause and effect relationship has been difficult. Therefore, cataract research has focused on understanding normal lens physiology and identifying those processes that are vulnerable to aging. It has long been recognized that lens transparency results from the very high concentration of soluble proteins, the crystallins, within a specialized lens fiber cell. It has also long been known that there is little turnover of proteins within these cells. An adult lens contains proteins synthesized at the earliest stages of embryological development, making fiber cell proteins especially susceptible to the effects of aging. During aging and cataract formation, soluble lens crystallins tend to coalesce into high molecular weight complexes that cause light to scatter. The normal lens counteracts this aggregation process through the function of α-crystallin, which acts as a molecular chaperone, preventing the unfolding and aggregation of proteins. Thus, one hypothesis for the formation of age-related cataract is that α-crystallin's chaperone function decreases as a consequence of age and exposure to environmental factors. This decreased chaperone function permits lens proteins to coalesce into light scattering aggregates, with subsequent opacification.

*Advance*: New research provides data linking the formation of high molecular weight crystallin complexes with diminished chaperone activity. Scientists examined lenses during aging and cataract formation. They found that as α-crystallin acts to prevent the deleterious effect of aggregate formation by binding to other proteins, α-crystallin itself becomes incorporated into an aggregate. Since α-crystallin strongly binds to the lens fiber cell membrane, it becomes a vehicle for complexes to accumulate at the membrane. Once these light scattering aggregates are bound to the membrane, there may be further damaging physiological effects that accelerate cataract formation. New data show that the α-crystallin in this membrane-bound aggregate has a significantly diminished capacity to function as a chaperone, indicating that its protective effect has been neutralized.

*Implications*: These new studies demonstrate a direct correlation between membrane bound α-crystallin, diminished α-crystallin chaperone function, and high molecular weight complex accumulation, suggesting a possible mechanism of cataract formation. This model suggests points for clinicians to intervene prior to the formation of a clinically evident cataract.

Cobb B, Petrash M: α-Cystallin chaperone-like activity and membrane binding in age-related cataracts. Biochemistry 41(2): 483-490, 2002.

## A Gene Expressed in Brain and Retina is Associated with Glaucoma

*Background*: Primary open angle glaucoma is a major public health problem and the number one cause of blindness in African-Americans. Approximately 2.2 million Americans have glaucoma, and approximately the same number may have the disease and not know it. Even though glaucoma was first described over 100 years ago, there is no complete understanding of its pathogenesis. The hallmark of glaucoma is a distinct pattern of optic nerve degeneration. This degeneration is most commonly associated with elevated intraocular pressure; however, in some patients an elevation in pressure is not evident on clinical examination. This complex clinical profile of the disease along with the lack of animal models that mimic the human disease make the study of glaucoma particularly difficult. In recent years, investigators have turned to genetic approaches to find clues as to the pathophysiology of glaucoma.

*Advance*: Scientists have recently identified a human gene, OPTN, that is linked to a disease known as "low-tension" glaucoma. In patients with this form of the disease, clinicians are unable to detect pathological elevations of intraocular pressure. Four separate mutations in this gene were identified in families in which "low-tension" glaucoma was known to be inherited. Further screening of glaucoma patients suggested that mutations in OPTN may be a risk factor for "low-tension" glaucoma patients. This gene encodes the protein optineurin, which is expressed in a number of tissues including the brain and retina. Optineurin has been shown to interact with other brain proteins such as huntingtin, the protein responsible for Huntington's disease and therefore may have a significant neurological function. Other studies suggest that optineurin participates in a signal transduction pathway involving tumor necrosis factor-alpha, a factor that is believed to increase the severity of optic nerve damage in glaucoma.

*Implications*: Glaucoma is a complex disease involving anterior segment tissue, retina, and the optic nerve. However, visual loss ultimately is due to degeneration of the optic nerve. Increasingly, scientists have viewed protecting the optic nerve as the key to treating the disease. The identification of OPTN as a "glaucoma" gene provides a tool to study the biochemical pathways leading to optic nerve degeneration, as well as giving insight into designing neuroprotective strategies.

Rezaie T, Child A, Hitchings R, Brice G, Miller L, Coca-Prados M, Héon E, Krupin T, Ritch R, Kreutzer D, Crick R, Sarfarazi M: Adult-onset primary open-angle glaucoma caused by mutations in optineurin. Science 295: 1077-1079, 2002.

## Regeneration: A Developmental Switch

*Background*: There is a pressing need to understand the mechanisms that allow nerve cells to regenerate following injury or disease. Unlike nerve cells in the peripheral nervous system, neurons in the central nervous system (CNS) have a limited regenerative capacity. Nerve cells typically have two types of extensions that arise from their cell bodies. Axons are normally quite long and extend over considerable distances. Dendrites are much shorter and extend very short distances from the cell body. The inability of CNS neurons to regenerate is largely due to the failure of their axons to re-grow. It is believed that the inability of CNS axons to regenerate is due to the presence of a non-permissive microenvironment, containing factors that inhibit regeneration. Although these may be important factors, nerve cell interactions may play an inhibitory role as well.

*Advance*: Recent work has shown that the developmental state of nerve cells may also play a role in the ability of CNS neurons to regenerate. It is known that postnatal retinal ganglion cells (RGC) in culture do not grow axons as rapidly as RGCs cultured from embryos. But researchers may now have identified a developmental switch that limits the ability of older RGCs to grow axons. They compared growth responses of cultured RGCs from embryonic rats and newborn rats using a wide range of conditions, including trophic and glial factors and different substrates. None of these conditions enhanced the ability of RGCs from newborn rats to accelerate the growth of their axons. These results suggest that the ability of neurons to grow axons may in part be due to an intrinsic factor and not dependent on factors in the microenvironment. Dissociated embryonic RGCs had a faster growth rate that did not slow down as these cells matured in culture. By contrast, dissociated postnatal RGCs cultured in explants in contact with other retinal cells had dramatically reduced axonal growth rates. It is possible that the change in growth rate could be signaled by other retinal cells. Additional co-culture experiments revealed that contact with developing amacrine cells signal RGCs to switch to a dendritic growth mode as the RGCs begin to acquire polarity. The contact between RGCs and amacrine cells not only stimulated the growth of dendrites, but also impaired the growth of axons. If this signal remains in effect long after development is finished it could suppress axonal regeneration in the adult.

*Implications*: This work demonstrates that interactions between developing CNS neurons can involve inhibitory factors, and that interneurons like amacrine cells may play a role in regeneration. It will be important to identify the intrinsic changes occurring during RGC maturation, and determine if they can be reversed. If CNS neurons are axotomized near the cell body, a new axon can regenerate from an existing dendrite. Mature RGCs respond in a similar way. It has also been shown that CNS neurons undergo an accelerated axonal growth rate in response to a second axon injury (conditioning lesions). The challenge remains to discover the signals that switch neurons back to the axonal growth mode. The present work suggests that a clearer understanding of the developmental switch from axonal to dendritic growth may be a key factor in CNS regeneration. Also, the role amacrine cells play in regulating RGC growth underscores the importance that interneurons may play in suppression of CNS regeneration.

Goldberg J, Klassen M, Hua Y, Barres B: Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. Science 296: 1860-1864, 2002.

## How are Sensory Representations Mapped in the Brain?

*Background*: How is the topography of the eye preserved as it connects to centers in the brain during development? This has long been a crucial question in the development of the central nervous system and its connections. The precise topography of the visual system provides an ideal model to address such problems. The chemoaffinity hypothesis has been put forward as a means to explain how the visual system develops a spatial representation of the visual world. It suggests that topographic projections of the retinal axons are regulated by chemical markers expressed in corresponding gradients in both the retina and central target structures of the brain. This hypothesis has led to the identification of many candidate molecules expressed in gradients on dorsal-ventral and anterior-posterior axes of the retina and targets in the brain.

*Advance*: Recent work in mice has shown that a particular class of ephrin receptors and ephrin ligands (proteins that cause nerve axons to either repel or attract each other), dictate how axons from ganglion cells in the developing retina map onto the visual centers of the brain. The resulting projection formed a topographic map along the dorsal-ventral axis. The A-class of ephrins were previously known to be important in mapping the left-right, or horizontal, projection of the eye into the brain. The current work demonstrated that another class of ephrins, the B-class, forms the top-bottom, or vertical, axis of the topographic map of the retina into the brain. Using a mouse ephrin mutant, this study found that retinal axons expressing B-class receptors are guided along the vertical axis of the retina to their appropriate termination points in the brain through interactions with their corresponding ephrins. The retinal projections of normal and ephrin mutant mice were analyzed by injecting fluorescent dyes that showed the axonal projections from the retina into the brain in its entirety. Researchers found that some vertical axis axons in mutant mice lacking proteins ephrinB2 and ephrinB3 mapped to incorrect targets in the brain. The study also showed that the vertical axis axons in normal mice terminated in correct targets through EphB/ephrin-B interactions. Future steps will be to determine the precise nature of the signaling system of these molecular interactions.

*Implications*: This research helps us understand how axons find the correct paths during development. It also provides us with insights into the role that attractive and repulsive mechanisms play during normal wiring of the brain. Much of our knowledge of the world is based on sensory representations mapped in the brain. This work provides us important clues on how these maps are formed and how the structural organization of the brain is established. Many neurological disorders involve inappropriate connections in the brain. Thus, this work may give a clearer understanding of connection pathways that will be essential to diagnose and treat neurological disorders in the future.

Hindges R, McLaughlin T, Genoud N, Henkemeyer M, O'Leary D: EphB forward signaling controls directional branch extension and arborization required for dorsal-ventral retinotopic mapping. Neuron 35: 475-487, 2002.

## The Process of Light Adaptation Involves the Physical Movement of Proteins

*Background*: Vision is perhaps the most fundamental of our senses. While all parts of the eye and many parts of the brain are important for vision, a most vital component is the retina. Remarkably, the retina has the ability to adapt to changes in light intensity that allows us to see over a very wide range of illumination. The process by which the visual system changes its sensitivity, depending on the ambient light level is called adaptation. The photoreceptor cells in the retina actually change their sensitivity. This is a slow process that may take many minutes until the visual system is fully adjusted to new light levels.

*Advance*: NIH-supported scientists have reported a new cellular mechanism of rod photoreceptor adaptation that is triggered by daylight levels of illumination. The mechanism involves a massive light-dependent translocation of the photoreceptor-specific protein, transducin, between the functional compartments of the rod. Up to ninety percent of the transducin molecules were moved from the rod outer segment, where light is transduced, to other cellular compartments. This transfer occurred on a time scale equal to tens of minutes. The reduction in the transducin content of the rod photoreceptor outer segment was correspondingly accompanied by a reduction in the amplification of the rod photoresponse.

*Implications*: The finding that transducin physically translocates in response to light is most significant, not only for the process of light adaptation, but possibly more widely for the process of phototransduction. Previously light adaptation and light transduction were thought to involve only processes of saturation of the involved proteins by light. This new finding shows that this is not the case for adaptation, and suggests protein translocation should be looked for in other steps in the process of signal transduction by photoreceptors.

Sokolov M, Lyubarsky AL, Strissel KJ, Savchenko AB, Govardovskii VI, Pugh EN, Arshavsky VY: Massive light-driven translocation of transducin between the two major compartments of rod cells: A novel mechanism of light adaptation. Neuron 33: 95-106, 2002.

## Molecules that Mediate Ocular Inflammation ("Uveitis") Are Identified

*Background*: Intraocular inflammatory diseases, pooled under the term "uveitis", are responsible for ~10 percent of vision loss in the USA. The majority of inflammatory processes are mediated by immune processes, initiated by lymphocytes that belong to the T-helper populations of type 1 (Th1) or type 2 (Th2). Th1 cells are responsible for "cell-mediated" responses, whereas Th2 cells initiate humoral and allergic reactions. Th cells exert their biological effect by releasing hormone-like molecules, designated "cytokines" that activate other cells and thus initiate the inflammatory process. A major component of this process is the recruitment of lymphoid cells into the affected site, via a mechanism termed "chemoattraction." The molecules that bring about chemoattraction are named "chemokines," a family of small proteins that are released by both lymphoid and a variety of resident tissue cells, and attract other lymphoid cells that carry receptors specific to the chemokines. Knowledge about the molecules responsible for ocular inflammation is essential to the development of therapeutic modalities, and the present study is the first comprehensive analysis of the molecules involved in inflammation of mouse eyes.

*Advance*: In this study researchers identified the major cytokines, chemokines, and chemokine receptors that are highly expressed in three animal models of ocular inflammation. The animal models were induced by (1) Th1 cells, (2) Th2 cells, or (3) by an autoimmune process, named "experimental autoimmune uveitis" (EAU), that closely resembles certain uveitic conditions in humans. Different molecules were found to be preferentially involved in inflammation induced by Th1 or Th2 cells, as detailed in the paper cited below. Importantly, the profile of molecules up-regulated in the pathogenic process of EAU closely resembled that of the Th1 cell-induced inflammation. Data collected in this study also revealed that normal eyes express constitutively high levels of three chemokines. The data suggest that these chemokines are responsible for the attraction of the primary inflammation-inducing Th cells into ocular tissues.

*Implications*: The comprehensive analysis of cytokines, chemokines, and chemokine receptors recorded in this study provides basic information about the expression of these inflammation-related molecules in the affected mammalian eye for the first time. Therapies targeted at these molecules have become available for treatment of various immune-mediated diseases. The findings of the present study underscore the multiplicity of molecules involved in the process of ocular inflammation and suggest that inhibition of sight-damaging ocular inflammation should be targeted at "upstream mediators", such as the cytokine designated Interleukin 1.

Foxman EF, Zhang M, Hurst SD, Muchamuel T, Shen D, Wawrousek EF, Chan C-C, Gery I: Inflammatory mediators in uveitis: differential induction of cytokines and chemokines in Th1- versus Th2-mediated ocular inflammation. J Immunol 168: 2483-2492, 2002.

## Motivational Control of Behavior

*Background*: When we learn to do a task, we are matching a voluntary movement to a stimulus condition according to a set of rules. However, a more fundamental reason for performing a movement is based on physical needs, such as a desire for food and water. These kinds of biological needs can be described as motivational, as opposed to cognitive, or rule-based, performance. Primates are equipped with neural circuits in certain areas of the brain that predict the availability of reward during the performance of behavioral tasks. It is not known, however, how reward value is incorporated in the control of action.

*Advance*: Scientists have identified neurons in an area of the monkey brain called the caudate nucleus that create a spatially selective response bias depending on the expected gain. In behavioral tasks, a monkey had to make a visually guided eye movement in every trial, but was rewarded for a correct response in only half of the trials. Reward availability was predictable on the basis of the spatial position of the visual target. We found that caudate neurons change their discharge rate systematically, even before the appearance of the visual target, and usually fire more when the contralateral position is associated with reward. Strong anticipatory activity of neurons with a contralateral preference is associated with decreased latency for eye movements in the contralateral direction. We conclude that this neuronal mechanism creates an advance bias that favors a spatial response when it is associated with a high reward value.

*Implications*: This study introduces a new perspective on the role of the basal ganglia in goal-oriented control of movement. In the past the focus has been on the role of dopamine neurons and their relation to learning rules and predicting reward. Our new results obtained from GABAergic caudate neurons shift the focus to the integration of motivation and action. The physiology of these neurons offers insights into the mechanisms that drive an organism toward satisfying basic biological needs. These findings shed new light on basal ganglia pathology such as Parkinson' s disease, akinesia, or abulia, where patients may be unable or unwilling to move. Investigators can use these results to identify target areas for the development of behavioral and neurochemical therapy.

Lauwereyns J, Watanabe K, Coe B, Hikosaka O: A neural correlate of response bias in monkey caudate nucleus. Nature 418: 413-417, 2002.

## An Unexpected Neuronal Specialization for Depth Perception

*Background*: When we look at a three-dimensional scene, the eyes fixate on one object at a certain depth. Images of that object project onto corresponding points of the retinas of both eyes. Other objects at different depths project to non-corresponding points of the retinas. These points create a binocular disparity; in effect, two different retinal images. These locations are related by a displacement along a horizontal axis, because the eyes are separated horizontally. Thus, finely spaced measures of horizontal disparity are required to detect variations in depth within the scene. It therefore seems natural to assume that horizontal disparities would be encoded by brain cells selective for binocular disparity. However, current understanding of neurons in the primary visual cortex suggests that different neurons signal disparities along many different axes, called their preferred monocular orientation.

*Advance*: In the past, disparity-selective neurons were tested with disparities applied along only one axis. Such tests cannot reveal whether or not neuronal responses are specialized to exploit the horizontal bias of naturally occurring disparities. To test this, scientists explored responses of single neurons to disparities applied along several axes, using a visual stimulus that is itself not oriented (such as random dot stereograms). Most neurons tested in this way modulated their firing rate over a wider range of horizontal disparities than vertical disparities, even if their preferred monocular orientation was not horizontal. This represents a specialization for the types of horizontal disparities caused by objects at different depths.

*Implications*: This demonstrates that the properties of visual neurons reflect the anisotropy of naturally occurring disparities, and that early processing is more complex than previously envisaged. Recent evidence indicates that activity in disparity-selective neurons of primary visual cortex does not directly support depth perception. This study provides the strongest evidence to date that these neurons nevertheless show a specialization for horizontal disparities, and thus may play a role in depth perception.

Cumming, BG: An unexpected specialization for horizontal disparity in primate primary visual cortex. Nature 418: 633-636, 2002.

## Identifying the Genetics of a Multiple Tumor Syndrome

*Background:* Carney complex (CNC) is a genetic syndrome associated with abnormal skin coloration that predisposes individuals to develop multiple non-cancerous tumors of the heart, skin, breast, nervous system, and endocrine glands (thyroid, pituitary, gonads, and adrenal). CNC is a rare disease, but its complicated symptoms and the involvement of many different organs of the human body suggest that the responsible genetic defects can offer insight into the basic function of all human cells. Also, by studying rare genetic diseases, scientists can learn useful information that can be applied to other, more common disorders. Identifying the CNC genes and understanding the process by which they promote tumor formation could help scientists develop useful clinical applications for other types of tumors.

*Advance:* Researchers studied the few families around the world that have CNC to identify the genes responsible for this disease. Their search was aided by using the information and tools of the Human Genome Project. The scientists identified two chromosomes that harbor the genes responsible for CNC, chromosomes 2 and 17. Chromosome 2 is involved in developing human tumors, and chromosome 17 harbors a gene known as *PRKAR1A* that is mutated in about half of the patients with CNC. After identifying the relevant genes from chromosomes 2 and 17, the researchers screened the genes for their possible involvement in non-cancerous tumors that occur in the same organs in the general population. They discovered that *PRKAR1A* is present in almost all human cells and is part of the structure of protein kinase A. Protein kinase A is a component of the most important signaling pathway, a route through which the external environment sends messages into the cell. In follow-up studies, the researchers showed that in its normal state, this subunit of protein kinase A appears to act as a tumor suppressor. However, when *PRKAR1A*'s action is eliminated, tumors of various organs develop.

*Implications:* Researchers now know that CNC can be caused by a *PRKAR1A* mutation. Furthermore, this research changes dramatically what scientists know about protein kinase A and its involvement in tumor development. Researchers can now focus their efforts on strategies to modify protein kinase A activity to develop treatments for patients with CNC as well as those with other nongenetic endocrine tumors.

Sandrini F, Matyakhina L, Sarlis NJ, Kirschner LS, Farmakidis C, Gimm O, Stratakis CA: Regulatory subunit type 1-α of protein kinase A (*PRKARIA*): a tumor-suppressor gene for sporadic thyroid cancer. Genes, Chromosomes, and Cancer 35: 182-192, 2002.

## New Mouse Model Mimics Rett Syndrome Features

*Background:* Rett Syndrome (RTT) is a developmental disorder that gradually robs apparently healthy infant girls of their language, mental functioning, and ability to interact with others. The genetic abnormality responsible for Rett syndrome involves the *Mecp*2 gene, located on the X chromosome. This defect prevents production of a protein called MECP2. When MECP2 is absent, excessive amounts of otherwise beneficial proteins are produced which causes harm to the brain. Males with RTT usually die before birth, and those who survive die early of a brain disorder. Females with RTT have more uncertain outcomes because they have two X chromosomes (males have one X and one Y). Therefore, females have two copies of every X chromosome gene, while males have only one. An altered *Mecp 2* gene in a male is likely to have significant adverse effects, since there is no normal "partner" to mitigate the effects of the abnormal gene. Very early in the development of a female fetus, one or the other X chromosome randomly gets inactivated in each cell. As these cells continue to divide in the developing female fetuses with RTT, the females end up with some cells with a normal *Mecp2* gene and some cells with an abnormal gene. As a result of the random selection of X chromosomes, females may have some or many of the features of the disorder, depending on the proportion of cells with the abnormal gene and the distribution of those cells. Researchers have been working to create an animal model that mimics many of the features of RTT. Previously, the researchers were unsuccessful at developing a model that consistently exhibited the symptoms of RTT.

*Advance:* Researchers recently developed a mouse model that improves on previous attempts. It more closely mimics physical and behavioral features of RTT by altering the *Mecp2* gene of male mice, without the complicating effects of X chromosome inactivation. Live born mutant male mice appear normal until 5 to 6 weeks of age, at which time they develop numerous and progressive neurological features such as body tremors and muscle abnormalities, spontaneous partial seizures, stereotypic forelimb motions, and a progressive, debilitating movement disorder. These mutant mice also exhibit behavioral features similar to RTT such as decreased activity, poor grooming, and high anxiety.

*Implications:* This animal model system is superior to previous attempts because it more closely mimics many of the features of RTT. The model will allow researchers to learn more about the natural progression of RTT, as well as test medications and other treatments that might prevent or lessen the symptoms of the disorder. Furthermore, understanding the molecular events underlying certain stages of this progressive disease may eventually lead to understanding some of the genes involved in regulating motor function, involuntary movements, seizures, and anxiety.

Shahbazian MD, Antalffy B, Armstrong DH, Zoghbi HY: Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. Human Molecular Genetics 11(2): 115-124, 2002.

Shahbazian MD, Young JI, Yuva-Paylor LA, Spencer CM, Antalffy BA, Noebels JL, Armstrong DL, Paylor R, Zoghbi HY: Mice with truncated MeCP2 recapitulate Rett syndrome features and display hyperacetylation of histone H3. Neuron 35: 243-254, 2002.

## Carriers of Fragile X Show Distinctive Characteristics

*Background:* Fragile X syndrome (FXS) is one of the most common forms of inherited mental retardation. It is caused by an abnormal number of repetitions in a three-nucleotide DNA sequence in one region of the fragile X mental retardation (*FMR1*) gene, which is located on the long arm of the X chromosome. Scientists don't know what causes a stable gene to become unstable and to expand to the full mutation (defined as more than 200 "repeats" of the nucleotide sequence). Full mutation of the FXS gene commonly causes abnormalities in development of the brain (resulting in mental retardation) and reproductive system. Full mutation also causes a characteristic physical appearance and hyperflexible joints. Although children with FXS show cognitive, behavioral, and physical characteristics that vary by sex, males who have the FXS mutation are more severely affected. Previously investigators did not associate distinctive characteristics with premutation carriers (generally considered to have between 55 and 200 "repeats"). However, recently scientists discovered that a subgroup of carriers exhibits some physical features of FXS or mild cognitive and emotional problems.

*Advance:* In three separate, but related, studies researchers found that 16 to 24 percent of female premutation carriers exhibit premature menopause, whereas women with a full mutation, or sisters of premutation carriers who carry 39 or fewer repeats, have an incidence of premature menopause of only 1 to 2 percent. Furthermore, the carrier women are likely to have inherited the premutation from their fathers. Additional research showed that a subgroup of premutation men are at risk for developing an apparently unique neurologic syndrome. This syndrome is characterized by tremors, muscle coordination problems, cognitive decline, generalized brain atrophy, impotence, nervous system disorders, mood changes, and mild Parkinson-like traits. This syndrome was not observed in full-mutation males.

*Implications:* Individuals with premutations of the *FMR1* gene are at risk of developing a distinct set of cognitive, behavioral and physical disorders. Since males with premutations and female premutation carriers are relatively frequent in the general population, cognitive and physical characteristics should be identified and the information translated into public health recommendations. Women of reproductive age who carry the premutation are not only at risk for having a child with FXS but are also at risk for premature ovarian failure. Similarly, fathers of premutation carriers may need to have their mutation status assessed to determine their risk for developing a neurologic disorder.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ: Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 57: 127-130, 2001.

Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ: Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain 125: 1-12, 2002.

Sullivan AK, Crawford DC, Scott EH, Leslie ML, Sherman SL: Paternally transmitted *FMR1* alleles are less stable than maternally transmitted alleles in the common and intermediate size range. Am J Hum Genet 70: 1532-1544, 2002.

## Harmless Virus Might Slow AIDS Progression

*Background:* The human immunodeficiency virus (HIV) infects the cells of the immune system by means of a "receptor complex." In a process analogous to a key fitting into a lock, molecules on the surface of the HIV cell bind to other molecules, called receptors, on the surface of immune cells, opening a pathway into the cell. Once the molecular "unlocking" takes place, the virus fuses with the cell and infects it. From past research, scientists have learned that variants of HIV exist. Some variants are present in greater numbers than others at the different stages of disease progression. Each variant uses different receptors on the immune cells to fuse with and enter the cell. For example, the HIV-1 variants that transmit infection and dominate the early stages of infection bind to a receptor known as CCR5. As the disease progresses, the virus is often replaced with other variants that bind to a different receptor, CXCR4. Recent studies of people with HIV show that some people who are also infected with certain other organisms show slower progression to full-blown AIDS. One such organism is the human herpesvirus 6 (HHV-6), a common virus that is apparently harmless in adults.

*Advance:* Scientists developed methods to maintain small blocks of living human tonsil tissue outside of the body and to infect the tissue with HIV-1 and HHV-6. Tonsils are part of the lymphoid system, the network of immune cells and molecules that police the body in search of disease. The researchers found that the HIV-1 variants that use CCR5 do no reproduce as fast in the presence of HHV-6 as they would in its absence; however, the HIV-1 variants that use CXCR4 receptors do not seem to be affected by the presence of HHV-6. The researchers also discovered the molecular mechanism by which HHV-6 affects HIV-1 reproduction. Tissues infected with HHV-6 produce large amounts of a molecule called RANTES. RANTES apparently blocks the CCR5 receptor, analogous to plugging up a lock with plumbers' putty. Therefore, by triggering RANTES production, HHV-6 deprives the early HIV variant of its binding site, which then prevents the virus from entering the cell. As added proof of this mechanism, the researchers added RANTES to cultures containing only the HIV variant that uses CCR5 and the virus reproduction was suppressed.

*Implications:* The finding shows how a harmless virus might be used to slow the reproduction of a lethal virus, such as HIV. Specifically, the research shows the molecular mechanisms that another microorganism uses to suppress the AIDS virus. If future studies in people show similar encouraging results, researchers may then be able to devise strategies for using the harmless HHV-6 to treat people infected with HIV.

Grivel J-C, Ito Y, Faga G, Santoro F, Shaheen F, Malnati MS, Fitzgerald W, Lusso P, Margolis L: Suppression of CCR5- but not CXCR4-tropic HIV-1 in lymphoid tissue by human herpesvirus 6. Nat Med 7(11): 1232-1235, 2001.

## New Insights into Immune System Function

*Background:* The immune system is made up of cells and tissues that recognize and attack foreign substances in the body. One component of the immune system, T-lymphocytes (T-cells) can tell the difference between molecules produced by the body (self-molecules) and foreign molecules. When T-cells recognize a foreign molecule, such as a virus or bacterium, they become activated and begin working to rid the body of the foreign invader. This is referred to as an immune response. Before T-cells can carry out this important task, the foreign molecule must bind to a receptor on the surface of the T-cell, in the same way that a key fits into a lock. The binding process triggers several chemical reactions, called signaling pathways, within the T-cell. These reactions enable the T-cell to attack the disease-causing substance. Two key signaling pathways in all cells that lead to this activation are the calcium pathway and the Ras pathway. Previously, researchers discovered that a protein called LAT (linker for activation of T-cells) coordinates the action between the calcium and Ras signaling pathways in T-cells for a normal immune response. Under abnormal conditions, T-cells can multiply out of control. When this occurs, they lose their ability to tell the difference between self-molecules and foreign molecules. Then they can attack the body's own tissues, resulting in an "autoimmune disease."

*Advance:* Researchers altered a single amino acid in the LAT protein in mice. The scientists observed that T-cell production was partially blocked in the mice at two weeks of age. However, by four weeks of age, the mice showed abnormal T-cells that had expanded rapidly. They also showed signs of autoimmune disease. The researchers discovered that the LAT protein produced by the mutated mice, while retaining the ability to connect T-cell activating receptors to the Ras signaling pathway, could not connect the receptors to the calcium signaling pathway. Without LAT and the calcium signal, the T-cells didn't develop normally and the immune response was altered.

*Implications:* This discovery not only provides important insight into how the immune system functions, but may lead to a better understanding of certain cancers of the immune system. The study also raises the possibility that some autoimmune diseases in humans result from mutations in the LAT protein that cause unbalanced or uncoordinated signaling in T-cells. According to the American Autoimmune Related Diseases Association, autoimmune diseases may affect up to 20 percent of the U.S. population and women are more likely to be affected than men. This research could lead to new ways to prevent and treat disorders in which the immune system attacks the body's own tissues.

Sommers CL, Park C-S, Lee J, Feng C, Fuller CL, Grinberg A, Hildebrand JA, Lacaná E, Menon RK, Shores EW, Samelson LE, Love PE: A LAT mutation that inhibits T cell development yet induces lymphoproliferation. Science 296: 2040-2043, 2002.

## A Large Number of Human Genes are Found to Contain Transposable Elements

*Background:* Transposable elements, or transposons, are long repetitive sequences of DNA that are embedded throughout the human genome in "silent" regions of chromosomes that do not make (code for) proteins. The human genome contains more than four million transposable elements. These sequences of DNA possess the ability to move around the genome, hence the name transposons or "jumping genes." Most of these moves are inconsequential because they do not affect regions of the genome that make proteins. However, occasionally transposons insert themselves in such a way as to cause genetic disease; one such example is factor VIII deficiency hemophilia. On the other hand, these jumping genes might be beneficial by increasing the functional versatility of the genome. Researchers have speculated that over a long evolutionary period, transposons might have contributed significantly to the divergence between primates and other mammals.

*Advance:* Researchers, in an attempt to find out how frequently transposons are embedded inside protein-coding regions without causing genetic disease, analyzed the coding regions of 13,799 human genes. The scientists found that 533 of them, about 4 percent, contained transposons or fragments of transposons. Extrapolating these findings to the entire human genome indicates that 1,200 human genes contain these transposable elements within their protein-coding regions.

*Implications:* This finding indicates that the insertion of transposons into protein-coding regions of genes may explain the high frequency of alternative splicing, or pasting, in human genes. Alternative splicing is important because a gene that can be spliced in more than one way can produce several different proteins, making it possible for the gene to perform more than one job. Genes with this increased functional versatility can give a survival advantage to the organisms that possess them. Thus, transposon insertion can become a driving force behind species divergence. This discovery also paves the way for experimental use of transposons in genetic engineering. Inserting transposons into genes to provide new sites for alternative splicing may produce novel proteins that researchers can then develop into new therapies for a variety of diseases.

Nekrutenko A, Li W-H: Transposable elements are found in a large number of human protein-coding genes. Trends in Genetics 17(11): 619-621, 2001.

## Vasectomy Does Not Increase Prostate Cancer Risk

*Background:* As many as one in six U.S. men older than 35 has had a vasectomy, approximately 500,000 vasectomies are performed each year in the United States, and more than 10 percent of U.S. couples use vasectomy as their primary form of birth control. According to the Centers for Disease Control and Prevention, prostate cancer is a leading form of cancer among men in the United States, second only to skin cancer. Although no biological explanation exists for why vasectomy might be associated with an increased prostate cancer risk, a few studies conducted in the early 1990s reported a moderately increased risk of prostate cancer among men who underwent vasectomy; other studies found no such increased risk. Because of this conflicting evidence, urologists have encouraged increased prostate cancer screening of vasectomized men and generally have discouraged vasectomies in men with a family history of prostate cancer.

*Advance:* This study was conducted in New Zealand primarily because that country has the highest vasectomy prevalence in the world coupled with mandatory reporting to the National Cancer Registry of all new cancer cases. The researchers reasoned that if a link exists between vasectomy and prostate cancer, it would most likely be revealed in the New Zealand data. More than 2,200 men were interviewed for this study, almost half of whom were newly diagnosed prostate cancer patients and all of whom were between the ages of 40 and 74 and had been married at some point in their lives. Telephone interviewers asked the men about several factors, including vasectomy, smoking and alcohol consumption, prostate-specific antigen testing, family history of cancer, and general sociodemographic characteristics. The study concluded that vasectomized men were no more likely to have prostate cancer than were men who had not had a vasectomy. The study also found no increased risk of prostate cancer among men who had had vasectomies 25 or more years before the interviews (38 percent of the men interviewed for this study) and no association between the risk of prostate cancer and history of smoking, consumption of alcohol in the past 5 years, number of children, and history of a sexually transmitted disease.

*Implications:* According to the results of this study, vasectomy does not increase the risk of prostate cancer, even after 25 years or more. In addition, men who reported having a father or brother with a diagnosis of prostate cancer had an increased risk of prostate cancer themselves, regardless of whether they had had a vasectomy. In view of the widespread use of vasectomy and the relatively common occurrence of prostate cancer, an association between the two would be of great significance. However, this study's finding of no increased prostate cancer risk associated with vasectomy should bring significant relief to a large number of men and their partners.

Cox B, Sneyd MJ, Paul C, Delahunt B, Skegg DCG: Vasectomy and risk of prostate cancer. JAMA 287(23): 3110-3115, 2002.

## Neuroimaging Useful in Understanding Dyslexia

*Background:* Developmental dyslexia (also known as reading disability) is a poorly understood but common disorder involving abnormalities in language and visual perception. Scientists know that reading ability is closely associated with the skills used to understand the sound system of language (called phonological processing). People with dyslexia have trouble with phonological processing as well as with visual motion processing (reaction to visual stimuli), both of which may contribute to their reading disorder. Functional magnetic resonance imaging (fMRI) is a powerful imaging tool that allows researchers to observe the brain in action. Researchers have used these neuroimaging techniques to find the areas of the normally functioning brain where language-specific processing occurs. Scientists have also used functional brain imaging to learn about normal reading acquisition and development which is crucial for understanding reading achievement, reading strategies, and individual variation in mastering the reading process. These techniques have never been used, however, to study dyslexia.

*Advance:* Researchers used fMRI to observe and compare the activity in certain parts of the brains of normal readers (the control group) and those of individuals with dyslexia, while they performed certain tasks. For example, the researchers tested the subjects while they performed visual motion processing and phonological processing tasks, such as sound segmentation, rhyme generation, and working memory of sound. All of these skills are fundamental to reading. The researchers showed that people with dyslexia are unable to use certain parts of the brain normally used when performing tasks involving knowledge of the sound structure of words. In addition to examining specific areas of the brain, investigators studied the entire brain to learn about visual motion processing. They discovered that when presented with visual stimuli, the normal readers showed activation in both hemispheres of the brain. The individuals with dyslexia showed significantly weaker activation in the areas of the brain associated with visual motion processing.

*Implications:* The neuroimaging techniques of fMRI are beneficial for identifying specific areas of the brain that play an important role in reading and for understanding how the brain functions when learning to read. These techniques will also increase our understanding about the relationship between various brain activities involved in reading and about how the brain works in individuals with dyslexia. As the technology advances and as the techniques become more refined, researchers will be able to understand the brain structures that underlie the ability to read and how to treat individuals with reading disabilities.

Joseph J, Noble K, Eden G: The neurobiological basis of reading. Journal of Learning Disabilities 34: 566-579, 2001.

Zeffiro TA, Eden G: The cerebellum and dyslexia: perpetrator or innocent bystander? Trends in Neuroscience 24: 512-513, 2001.

## A Possible Gene for Childhood Language Disorders

*Background*: Children who fail to develop language normally (in the absence of factors such as neurological disorders, hearing impairments, or lack of adequate opportunity) are described as having specific language impairment (SLI). Although some children with SLI will successfully learn to compensate when they become adults, many do not. SLI has a prevalence of approximately 7% in children entering school and is associated with later difficulties in learning to read. Research studies have consistently demonstrated that SLI occurs in families, indicating that genetic factors are important in causing SLI.

*Advance*: NIH-supported scientists are scanning the genome for the location of the gene suspected of causing SLI, by studying families with members characterized with language/reading disorders. The study showed significant evidence of a link between chromosome 13 and susceptibility to SLI. Further analysis also suggests two additional gene locations on chromosomes 2 and 17 that may play a role in SLI. In addition, mutations in the same region in chromosome 13 is implicated in autism, and some children with autism show language deficits that are very similar to SLI.

*Implications*: This study indicates that using family and molecular genetic studies may reveal important genetic factors in finding genes for complex language disorders, such as SLI.

Christopher W Barlett, Judy F Flax, Mark W Logue, Veronica J Viel, Anne S Bassett, Paula Tallal, Linda M Brzustowitz: A Major Susceptibility Locus for Specific Language Impairment is Located in 13q21. Am. J. Hum. Genet. 71: 45-55, 2002.

## Rapid Renewal of Auditory Sensory Stereocilia Aid Recovery to Hearing Loss

*Background*: Stereocilia, or hair cell bundles, are fine projections in the inner ear that vibrate when stimulated by sound. The movement of the stereocilia results in a molecular pathway that generates an electrical signal from the auditory nerve to the brain, where the energy is distinguished as sound. Stereocilia are located in the surface of the inner ear and are supported by a rigid and dense core of filaments. Until recently, this core was thought of as a stable structure whose sole function was to serve as rigid supports for changes in the mechanical constitution of the hair cells. Recent studies have shown that the stereocilia core has additional roles.

*Advance:* NIH intramural scientists have discovered that there is a continuous renewal of the stereocilia core every 48 hours. This process occurs in the mature bundles during recovery from temporary noise induced hearing loss and suggests that the stereocilia core structure plans an unforeseen role in this recovery process.

*Implications:* Recognition of this dynamic aspect of stereocilia is essential to the understanding of the development and maintenance of normal sensory function and sheds new light on the unique properties of hair bundles. Such a renewal mechanism could also provide more information on the molecular basis of genetic, environmental and age-related inner ear disorders that involve malformation or disruption of stereocilia.

Schneider ME, Belantseva I, Kachar B: Structural cell biology: Rapid renewal of auditory hair bundles. Nature 418: 837-838, 2002.

## A Sound Transduction Motor Protein Facilitates the Speed of Sound

*Background*: The sensory hair cells in the inner ear of mammals function as the mechanical transmitters of sound. Stereocilia are formed by cone-shaped bundles of sensory hair cells. The movement of the stereocilia initiates the complex pathways of molecular signals that stimulate the auditory nerve which carries information to the brain, eventually becoming the sensory perception of sound. One important component in this pathway is Myosin-1C, a major motor protein involved in the movement of the stereocilia. It is hypothesized that motor proteins serve as the link between the stereocilia's membrane channels and cell core thereby initiating cell depolarization following sound vibration.

*Advance*: NIH-supported scientists are in the process of deciphering how Myosin-1C works. Specifically, they used a chemical-genetic approach to inhibit Myocin-1C motor protein activity in mice by introducing a custom designed amino acid that alters the protein's function, and prevent the events required to power the motor activity of the stereocilia. The designer amino acid rendered the protein susceptible to a controllable inhibitor, thus, allowing managed regulation of the protein's motor function. Myosin-1C with a custom amino acid shows diminished function in the presence of inhibitor, but functions normally in the absence of inhibitors. Wild type Myosin-1C functions normally with and without inhibitor.

*Implication*s: These results demonstrate the importance of Myosin-1C in sound transduction, allows observation of protein function in a controllable native environment and permits assessment of protein function in a biological process. The findings are invaluable to furthering the development of targeted therapeutic treatments for individuals with hearing impairment and provides alternatives and variations to gene therapy methods. Future treatment strategies will rely on the results of further research on signaling pathways and effector components as means to decipher the molecular properties of mechanoelectrical sound transduction.

Holt JR, Gillespie SK, Provance DW, Shah K, Shokat KM, Corey DP, Mercer JA, Gillespie P: A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells. Cell 108: 371-381, 2002.

## Discovery of an Amino Acid Taste Receptor

*Background*: Taste is responsible not only for attraction and repulsion to various foods but is also responsible for providing important information about the chemical environment. The basic taste qualities are sweet, sour, salty, bitter and umami (the taste of monosodium glutamate or the taste associated with protein-rich foods). A major challenge in taste research is identifying the various types of taste receptors on the tongue that respond to different structurally diverse tasting compounds. Recently, scientists have identified a taste pathway dedicated to tasting amino acids, the building blocks of proteins that are involved in the biological processes in the body.

*Advance*: It has been known that sweet-, bitter- and umami-tasting substances activate G-protein–coupled receptors in the tongue. Recently, NIH-supported scientists have shown that G-protein-coupled receptors that respond to sweet compounds can alter their selectivity to sweetness by combining receptor subunits. The scientists discovered that two subunits in the T1R family, T1R1 and T1R3, can combine to form a broadly tuned L-amino-acid receptor, T1R1+3, that responds to most of the 20 standard amino acids. The receptor is not sensitive to natural or artificial (i.e., non-amino acid) sweeteners. The T1R1+3 receptor is also responsive to monosodium glutamate, the basic ingredient in umami taste. In contrast, the T1R2+3 receptor is a sweet receptor that does not respond to L-amino acids. These results indicate that the subunit partner to T1R3 (either T1R2 or T1R1) determines whether a receptor will be a sweet or amino acid receptor.

*Implications*: Identification of an amino acid taste receptor provides a new tool to help scientists decode the molecular basis for detecting different taste qualities. Fish have been long known to possess taste receptors in the oral cavity and along the external surface of the body that respond to specific amino acids. This is the first study that shows a comparable sensitivity for amino acids also exists in mammals.

Greg Nelson, J Chandrashekar, MA Hooh, L Feng, G Zhao, NJP Ryba, CS Zuker: An amino-acid taste receptor. Nature 416: 199-202, 2002.

## Signaling Pathway Regulates Pillar Cell Development in the Inner Ear

*Background*: In mammals, sound is perceived in the organ of Corti of the cochlea. It is an area of the inner ear that contains sensory hair cells. One of the most striking aspects of the organ of Corti is the arrangement of both sensory hair cells and other cell types in a very regular cell pattern. In particular, the hair cells are arranged in a single row of inner hair cells and three or four rows of outer hair cells. Moreover, the row of inner hair cells are separated from the rows of outer hair cells by a space referred to as the tunnel of Corti. The boundaries of the tunnel of Corti are formed by two rows of specialized pillar cells. Recent studies have shown that normal development of pillar cells and the tunnel of Corti are required for normal hearing to occur. However, despite the importance of the pillar cells in the auditory process, the molecular pathways that are required for their development have not been determined.

*Advance*: NIH intramural scientists have demonstrated that the fibroblast growth factor (FGF) signaling pathway plays a key role in regulating pillar cell development. FGFs are secreted molecules that influence several different developmental events by binding to specific FGF receptors on cell surfaces. The scientists determined that a specific FGF receptor, FGFr3, is turned on in cells that develop into pillar cells. In addition, the developing inner hair cells located next to the pillar cells turn on a specific fibroblast growth factor, FGF8. Therefore, both FGF and its receptor are expressed in a pattern that is consistent with a role in pillar cell development.

To begin to examine the role of the FGF signaling pathway, the activation of FGFr3 receptor was blocked using a specific FGFr antagonist. Inhibition of FGFr3 led to an inhibition of pillar cell development. However, if FGFr3 and pillar cell development was partially inhibited FGFr3 for only a brief period of time, then pillar cell development resumed, suggesting that continuous FGF signaling is required for normal pillar cell development. To determine the effects of increased activation of FGFr3, a strong activator of this receptor, FGF2, was added to the developing cochlea. Addition of FGF2 led to an increase in the number of cells that develop into pillar cells. This effect is dependent on the amount of FGF2 added and on the timing of its addition. As the organ of Corti matures, the effects of FGF2 are reduced.

*Implications*: These results demonstrate that the FGF signaling pathway plays a key role in regulating the development of cells as pillar cells. The results also provide valuable insights into the mechanisms that ensure the development of a normal organ of Corti. Its likely that pillar cells grow directly adjacent to the inner hair cells because the hair cells serve as the source of FGF. Since broad activation of FGFr3 can lead to an increase in the number of pillar cells, it seems likely that pillar cells may be regulated by a limited amount of FGF within the inner ear. Since the source of FGF is the inner hair cells, pillar cells will always develop adjacent to them. Future experiments will examine the specific role of inner hair cells in pillar cell development as well as exploring other mechanisms that may exist to ensure that only a single tunnel of Corti develops.

Mueller KL, Jacques BE, Kelley MW: FGF Signaling Regulates Pillar Cell Development in the Organ of Corti. J Neurosci.22(21): 9369-9377, 2002.

## Bacterial Biofilms Make Ear Infections Tough to Overcome

*Background:* Infection or inflamation of the middle ear (otitis media) occurs at least once in 75 percent of children by their third birthday. Approximately half of these children will have an average of three or more ear infections during their first three years of life. Otitis media (OM) is the most common reason for a child to receive antibiotics and to undergo general anesthetic. In the United States this represents an annual medical cost and lost wages of approximately $5 billion.[5]

*Advance:* While the socioeconomic impact of the disease is clear, the factors that cause it remain unclear. NIH-supported scientists are studying the specific molecular mechanisms that allow bacteria in conjunction with viruses to cause OM. While the three primary bacterial causes of OM have been identified, *Haemophiles influenzas, Streptococcus pneumoniae* and *Mirabella catarrhalis*, how these organisms interact with the middle ear environment to induce OM symptoms is unclear. Recent findings highlight certain physical and environmental features of a microscopic organisms – or mucosa biofilm – that enables bacteria to be more resistant than free-living bacteria of the same species. These mucosal biofilms exist as large congregations of organisms growing on the mucosal surface of the middle ear and are believed to be the contributing factor to chronic OM. Organisms that are part of a biofilm display altered physical, biochemical and physiological characteristics such as reduced metabolic rates and altered gene expression. The reduced metabolic rate makes organisms resistant to antimicrobial drugs, and the physical structure of the biofilm provides a shield against the body's immune system, thus providing an incredibly resilient environment for the invading microbes. In addition, longtime exposure to biofilms may result in bone damage. Identifying the process that lead to OM is critical for developing preventative treatment such as vaccines and other strategies designed to block the infectious process.

*Implications*: Analysis of the microscopic array will be critical in providing information necessary to understand the OM disease process. This information will lead to new molecular approaches for prevention and treatment of OM.

Erlich GD, Veeh R, Wang X, Costerton JW, Hayes JD, Hu FZ, Daigle BJ, Ehrlich MD, Post JC: Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. JAMA 287(13):1710-1715, 2002.

Jung JY, Chole RA: Bone Resorption in Chronic Otitis media: The Role of the Osteoclast. ORL J Otorhinolaryngol Relat Spec. 64(2): 95-107, 2002.

Giebink GS, Bakaletz LO, Barenkamp SJ, Eskola J, Green B, Gu XX, Harada T, Heikkinen T, Karma P, et. al.: Recent advances in otitis media 7 Vaccine. Ann Otol Rhinol Laryngol Suppl111: 82-94, 2002.

Ogra P, Barenkamp SJ, DeMaria TF, et. al.: Recent advances in otitis media 6 Microbiology and immunology. Ann Otol Rhinol Laryngol Suppl 111: 62–81, 2002.

Ryan A, Bakaletz LO, Juhn SK, Jung TT, Li JD: Recent advances in otitis media 5 Molecular biology and biochemistry. Ann Otol Rhinol Laryngol Suppl 111: 52-61, 2002.

[5]National Institutes of Health: Table - Cost of illness and NIH support for selected diseases and conditions. Disease-Specific Estimates of Direct and Indirect Costs of Illness and NIH Support, 1997.

## You Can Teach an Old Owl New Tricks: Plasticity in the Adult Brain

*Background*: Someone calls your name and you turn to look at who it is – a process so automatic it seems simple. But is it? Our ability to localize the source of sound relies on complex computations in the brain which translates auditory localization cues into representations in space. Sound localization cues result from the interaction between the ears, eyes, brain and the incoming sound. Although many animals can localize sound soon after birth, the exact relationships between localizing cues differences in sound volume or time of sound arrival between the two ears and locations in space are shaped and modified by experience.

*Advance*: The sound localization pathway has become a model system for studying mechanisms by which the nervous system learns from experience. The barn owl, a nocturnal predator with keen vision and hearing and a highly evolved capacity for sound localization, has been studied extensively. NIH-supported scientists have demonstrated that a part of the brain (optic tectum) is the source of a visually-based instructive signal that calibrates auditory information as a map of visual space. When barn owls are raised wearing special prism spectacles that displaces their field of vision, another part of the brain, the external nucleus of the inferior colliculus (ICX), adapts by also shifting the auditory space map according to the optical displacement caused by the prisms. Topographic visual activity in the optic tectum could serve as the template that instructs changes in the auditory space map.

Additional experiments were conducted to investigate the mechanisms of adaptability (plasticity) in the owl's midbrain. Plasticity in the central nervous system that involves learning is generally more restricted in adults than in young animals and the sound localization pathway has been shown to be far more limited in its ability to adjust to abnormal experience in adult versus juvenile barn owls. In experiments using adult and juvenile owls wearing prism spectacles, it was shown that juveniles learn new associations between auditory cues, such as the time difference it takes sound to reach each ear (interaural time difference or ITD), and locations in visual space; and the young owls acquire new neurophysiological maps of ITD in the optic tectum. Adults owls do neither. However, when the prismatic shift is experienced in small amounts over time, ITD maps in adults owls do adapt. In addition, once the adult brain learned to adapt to the shifts through incremental training, new ITD maps were reacquired when the adult owls were given the spectacle at a later time. These results demonstrate a substantially greater capacity for plasticity in adult brains than was previously recognized, and a principled strategy for tapping this capacity that could be applied in other areas of the adult central nervous system.

*Implications*: By studying how the auditory and visual systems exerts its influence on sound localization pathway reveals some of the mechanisms by which the nervous system instructs such changes. In addition, studies on the plasticity of the brain may lead to new methods for teaching normal and learning disabled children as well as develop therapeutic strategies for restoring function to individuals who suffered brain injury or disease.

Hyde PS, Knudsen EI: The optic tectum controls visually guided adaptive plasticity in the owl's auditory space map. Nature, 415: 73-76, 2002.

Knudsen EI: Instructed learning in the auditory localization pathway of the barn owl. Nature 417: 322-328, 2002.

## Loss of Sex Discrimination and Male-Male Aggression

*Background*: Animals have evolved specific communication strategies to help them identify and attract a mate. Pheromones are a discrete class of chemical cues that signal the sex and the social status of a species and promote coordinated motor programs and physiological changes essential for breeding and aggression among animals of the same species. The highly reproducible and species-specific response to pheromones offers a valuable experimental system for studying the neural basis of genetically pre-programmed behaviors.

*Advance*: NIH-supported scientists are studying signal processing in receptor cells of the vomeronasal system in mice. The vomeronasal system (VNO) is an independent component of the olfactory (smell) system and responds to species-specific pheromones that elicit a variety of basic social and reproductive behaviors. The VNO signal transduction involves transient receptor ion channels and pathways which are activated by a protein called TRP2. The scientists removed genes of the TRP2 ion channel, which eliminated physiological activation of vomeronasal neurons by urine pheromones. This resulted in the failure of the mice to display typical pheromone-evoked aggression towards male intruders and inappropriate courtship behavior. Mating was not affected, but the mice without the TRP did not discriminate between male and female mice.

*Implications*: The results of this study demonstrate that the loss of a single ion channel type has widespread reproductive and other behavioral consequences, and that a major function of the VNO is to ensure the gender specificity of male mouse behavior by providing the brain with sensory cues that are essential for sex discrimination. In addition, the research raised further questions into the function and the development of the VNO pathway, including how sexual identity is processed by the brain and controls animal behavior, when is recognition of sex acquired by the brain, and what are the respective roles of genetic and extrinsic determinants in the developmental process.

Stowers L, Holy LE, Meister M, Dulac C, Koentges G: Loss of Sex Discrimination and Male-Male Aggression in Mice Deficient for TRP2. Science 295: 1493-1500, 2002.

## A Critical Period for American Sign Language Processing

*Background*: Signed languages such as American Sign Language (ASL) are natural languages similar to spoken languages, and thus enable scientists the opportunity to examine the effects of language structure and the manner which the brain processes and organizes language. It has long been known that the left side of the brain is involved with learning spoken language. However, scientists have learned that users of ASL utilize the right hemisphere (RH) as well as the left hemisphere (LH) of the brain.

*Advance:* NIH-supported scientists are examining the effect that age at which language is learned has on the parts of the brain involved in language processing. Scientists are studying bilingual individuals that know two languages that differ in both their structure and their modality: an audible-oral language, (English), and the a visual-manual language (American Sign Language). The unique demands of processing ASL may use certain parts of the right side of the brain not known for processing spoken languages. However, the ability for the right side of the brain to learn ASL may change with age, suggesting that a "critical" or "sensitive" period in development exists when ASL is learned early in life and brain regions can be activated for processing signed language.

The scientists used functional magnetic resonance imaging (fMRI) to compare brain activation of two groups of individuals who were fluent in spoken English and ASL. Although all subjects were native learners of spoken English, one group learned ASL from birth from deaf parents whose primary language was ASL, and the other group learned ASL in early adulthood (after puberty). The scientists observed that certain regions within the right side of the brain were activated by ASL even after puberty, however, the magnitude of these activation were less than in early learners of sign language. Early signers showed strong activations in one region of the right hemisphere, the angular gyrus (AG), but this region did not show significant activation in the late signers. It seems that there is a critical period during development that incorporates the AG for processing ASL.

*Implications*: The results reveal that exposure to a language that makes extensive use of hand, arm and facial movements can lead to a specialized type of language processing by the AG but only if that language is learned early in life. This region of the brain may possess some biological bias toward the processing of human motion, shape and location information, which enables it to be specialized for processing a spatial language such as ASL. The AG appears less susceptible to change after puberty, and thus, for signed language as well as other natural languages, the nature and timing of language input have significant effects on the identity and configuration of the language systems of the brain.

Newman AJ, Bavelier D, Corina D, Jezzard P, Neville HJ: A critical period for right hemisphere recruitment in American Sign Language processing. Nature 5(1): 76-81, 2002.

## Do Stutterers Have Different Brains?

*Background:* People who stutter are often subjected to emotional pain and social stigma due to their dysfluency. An individual who stutters may be more self-conscious of how to produce the sounds of speech, leading many to believe that anxiety or emotional problems are the causes for stuttering. There is no single known cause of persistent developmental stuttering (PDS). Recently, scientists began studying the neurobiology of stuttering in the cortical speech-language areas of the brain of individuals who stutter.

*Advance:* NIH-supported scientists performed brain imaging studies on two groups of adults; those with or without PDS. The scientists performed quantitative and qualitative measurements of brain imaging scans on these two groups. Results of the analysis showed that differences in the speech-language areas of the brain are more common in adults with PDS, although no one anatomic feature accounted for the group differences. The major anatomic finding was that the size and symmetry of the planum temporale (PT) differed significantly between the two groups. Both right and left PT size was significantly larger in the adults with PDS. The PT is important for higher order processing of language information. These findings may be functionally relevant in view of the effects of delayed auditory feedback on dysfluency and fluency of speech in individuals who stutter and those that do not. Delayed auditory feedback is a means of changing the speech of a speaker so he or she hears it with a delay. This method can sometimes make a dysfluent speaker more fluent. The motor control theory of speech production suggests that there are two main feedback loops, an outer "linguistic" loop and an inner "phonatory" loop. Thus stuttering can be modeled as a momentary instability in these systems when the timing between these two loops is interrupted.

*Implications*: The results about the PT size and other findings, such as differences in infolding patterns of the brain, demonstrate that atypical size or shape of the speech-language area may put individuals at risk for stuttering. The research suggests that these abnormalities permit normal development of language, but can cause abnormalities in the motor output of language, namely speech. The study of abnormal anatomy of the brain can provide a basis for future research on the severity of stuttering, brain imaging, as well as a model for the speech-motor control system.

Foundas AL, Bollich AM, Corey DM, Hurley M, Heilman KM: Anomalous anatomy of speech-language areas in adults with persistent developmental stuttering. Neurology 57: 207-215, 2001.

## Study Suggests Link Between Periodontal Disease and Heart Disease

*Background:* Atherosclerotic coronary artery disease currently contributes to half of all deaths in the United States. Yet, according to the latest data, more than one-third of all patients who die from this form of heart disease have no classic risk factors. Many recent studies suggest possible associations of atherosclerotic heart disease and infectious agents, including the bacterium *Porphyromonas gingivalis*, or Pg. This much-studied oral pathogen is strongly associated with the most frequent form of periodontal disease in the United States, adult perodontitis. However, this hypothesis remains controversial, largely because no direct casual relationship between Pg and atherosclerosis has been reported in the peer-reviewed medical literature.

*Advance:* A team of NIH grantees have demonstrated in animal studies that long-term, systemic exposure to Pg, in association with other classic risk factors, can accelerate the development of atherogenic plaques. The scientists note that their data appear to be the first example of such a finding in a living organism. In their study, the scientists fed two colonies of young mice either regular or high-fat diets. They then randomly inoculated subsets of mice in both groups with live Pg, or a diluted medium, once per week for 24 weeks. By the end of the study, the researchers found that atherosclerotic lesions of the proximal aortas and arortic trees appeared far earlier and were more advanced in the Pg-challenged mice than in those were not inoculated with the bacterium.

*Implications:* Though the hypothesis remains unproven, these experiments provide a model to explore in greater detail the possible synergistic effect of systemic infectious exposure with genetic and environmental factors that might promote atherosclerotic disease. Continued investigation in this area will answer important research questions that could have broad public health implications.

Li L, Messas E, Batista EL, Levine RA, Amar S: *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. Circulation 105: 861-867, 2002.

## Two Newly Identified Genes Offer Broad Insights into Causes of Cleft Lip and Palate

*Background:* Thousands of infants are born each year in the United States with disfiguring cleft lip and palate. For parents, though the condition is usually correctable after several surgeries, the diagnosis places a tremendous emotional and economic burden on their families. In an attempt to understand the causes of these disorders and perhaps one day prevent them, several NIH-supported research groups continue to search for genes that, when altered, contribute to these malformations. To date, approximately 15 genes already had been identified that are involved in human orofacial clefts. These discoveries provide the first solid clues for scientists to begin to define the biological causes of the extremely intricate, highly synchronized process of orofacial development.

*Advance:* This year, a team of NIH grantees and their collaborators reported the discovery of a gene called IRF6 that causes Van der Woude syndrome, one of the most common forms of syndromic cleft lip and palate. It is estimated that 30 percent of all cleft lip and palate cases are syndromic, meaning those affected inherit their orofacial malformations with other developmental abnormalities. Currently, there are an estimated 300 medically recognized forms of syndromic cleft lip and palate.

Another team of NIH grantees reported that a previously identified gene (PVRL1) implicated in a rare form of syndromic cleft lip and palate also seems to be involved in some cases of the non-syndromic disorder. The scientists, who identified the PVRL1 gene last year in studies on a remote Venezuelan island, reported that the most frequent mutation in the gene is also relatively common in a neighboring community with an unusually high rate of the non-syndromic cleft lip and palate.

*Implications:* One of the drawbacks of genetic studies is that they tend to focus on individual families, not on large genetically diverse populations. This means many gene discoveries are difficult to extrapolate to the general population. However, the IRF6 and PVRL1 genes seem fairly unique in that they appear to have broader applicability. Van der Woude syndrome closely resembles non-syndromic forms of the condition, raising the hope that future studies of the IRF6 gene will reveal important biological information about orofacial development in general. Interestingly, the scientists already have determined that people with another, more severe syndromic form also inherit mutations in IRF6, though in a different part of the gene than those implicated in Van der Woude syndrome. This suggests that different active sites of the IRF6 protein might have different functions during development. Until these and other genes were identified, such critical biological clues were extremely difficult, if not impossible, to identify.

S Kondo, BC Schutte, RJ Richardson, BC Bjork, AS Knight, Y Watanabe, E Howard, RLL Ferreira de Lima, S Daack-Hirsch, A Sander, DM McDonald-McGinn, EH Zackai, E Lammer, Aylsworth, H Ardinger, Bpober, D Moretti-Ferreira, A Richieri-Costa, MJ Dixon, JC Murray, C Houdayer, Mbahuau : Interferon Regulatory Factor 6 is essential for Orofacial, Skin and Genital Development. Nat Genet *in press*

Sozen MA, Suzuki K, Tolarova MM, Bustos T, Fernandez Iglesias JE, Spritz RA: Mutation in PVLR1 is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. Nat Genet 29: 141-142, 2001.

## Gene Identified as Causing Ectodermal Dysplasia Syndrome

*Background:* Ectodermal dysplasia syndromes, or EDS, are characterized by complete or partial loss of function of at least two tissues derived from the ectoderm, one of the original layers of cells that form before a developing baby is large enough to be seen. These tissues include: the buds that erupt into teeth, the follicles that produce hair, and the glands that produce sweat to cool the body. Of the more than 150 clinically recognized forms of EDS, the most common is the hypohidrotic form (HED), which indicates that a person has a diminished capacity to sweat.

Since Charles Darwin first described HED in the 1860s, scientists have had very little to work with to tease out the syndrome's molecular underpinnings. However, beginning in the 1950s, geneticists created three different mouse strains, all of which had patchy hair and abnormal tooth development that is reminiscent of HED. By 1998, NIH grantees cloned one of these genes in people called Eda. This gene is the most common cause of the syndrome and encodes a secreted protein that activates a specific protein receptor displayed on the surface of undifferentiated ectodermal cells. The same NIH grantees and colleagues thereafter isolated the second gene in humans and mice. This gene, called Edar, encoded the very receptor to which the Eda protein binds to trigger the developmental signal that goes awry to cause HED.

*Advance:* The above-mentioned NIH grantees and colleagues reported the isolation of the human version of the third mutant mouse gene. The gene's protein product functions as an adaptor protein in the same signaling pathway activated by Edar receptor upon binding Eda. An adaptor protein acts as a platform to recruit other proteins to an activated receptor, influencing where, when, and how signals are transmitted through the cell. The gene was identified based on studies with a large Middle Eastern family with a history of HED.

*Implications:* The latest gene discovery may help to explain the variability of HED in people. As the scientists noted, the adaptor protein contains structural regions that interact with different pathways, suggesting that the protein has multiple signaling outputs. The discovery also provides additional clues to understand the normal development of teeth and other ectodermally-derived tissues. Interestingly, the scientists stated that a confluence of evidence suggests that this developmental pathway, which was unknown prior to their gene discoveries, is shared among many organisms and might be at least 500 million years old. The pathway seems to control the early development of hair follicles and teeth in mammals, scales in fish, and, more speculatively, feathers in birds.

Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT, Zonana J, Overbeek PA: Gene defect in ectodermal dysplasia implicates a death domain adaptor in development. Nature 414: 913-916, 2001.

## Fundamental Discovery into Control of Motor Impulses

*Background:* When the brain transmits a motor impulse to the hands or feet, neurons release the chemical acetylcholine into the neuromuscular junction, the synapse between neuron and muscle cell. The acetylcholine binds to a receptor on the muscle cell, prompting the cell to contract, or move. For the movement to continue, the bound acetylcholine must be removed immediately to free their receptors for continued cycles of acetylcholine attachment and muscle contraction. The job of removing acetylcholine from its receptor belongs to an enzyme called acetylcholinesterase, or AChE. Though scientists know how AChE chemically removes acetylcholine, they have never explained how the enzyme localizes to the synapse in the first place. Does AchE act alone? Or, does another protein mediate its attachment to the acetylcholine receptor?

*Advance:* NIH scientists and their collaborators demonstrated that AChE must interact with the structural protein perlecan to function in the neuromuscular junction. Perlecan is embedded in the synaptic basil lamina, part of the protective extracellular matrix that surrounds muscle cells. The NIH scientists reached their determination based on studies with a novel mouse model that could not produce perlecan. They found, after extensive molecular analysis, that, though AChE was normally expressed in these mice, the enzyme did not also localize with the acetylcholine receptor. This indicated that perlecan is essential for AChE to attach to the basil lamina and perform its function.

*Implications:* This work provides the first conclusive evidence to explain how AChE localizes to the neuromuscular junction, key information involving a process that is critical to human health and well being. These studies also indicate a mechanism whereby constituents of the extracellular matrix can be inserted and turned over to facilitate muscle contraction. The scientists also note that their work offers an important molecular clue to explain in greater detail the rare Schwartz-Jampel syndrome and chondrodystrophic myotonia. Recent data indicate that these conditions stem from mutations in the perlecan gene.

Arikawa-Hieasawa E, Rossi SG, Rotundo RL, Yamada Y: Absence of acetylcholinesterase at the neuromuscular junction of perlecan-null mice. Nat Neurosci 5(2): 119-123, 2002.

## Studying Biology in its Natural Three Dimenional State

*Background:* As a human tissue forms, cells must anchor themselves to a grid of structural proteins known as the extracellular matrix. If the adhesion of cell to matrix is improper, serious health and/or developmental disorders can arise from improper cell attachment, migration, growth, and differentiation. Given its fundamental importance in human biology, cell-matrix adhesion has been studied for many decades. However, due to technical limitations, most data on the subject result from experiments in tissue culture, a flat, two-dimensional surface. This has raised the possibility that much of our current knowledge does not adequately reflect the true physiological behavior of cells in their normal, three-dimensional space, a potential problem as science and technology in the 21st century attempt to engineer replacement tissues.

*Advance:* A team of NIH scientists recently characterized the behavior of fibroblast cells grown on novel three-dimensional matrices derived from tissues or cell culture, materials comparable to that of a 2-D environment. The team found that the 3D adhesions differed significantly from the traditional focal or fibrillar adhesions in 2D space. The cells grown on the 3D matrices produced a different collective profile of proteins commonly associated with growth and adhesion, and they manifested differences in biological activity, structure, localization, and function.

*Implications:* This research establishes the importance of three-dimensional interactions of cells with their microenvironments in tissue culture and in animals. The novel 3D matrix mimics natural matrices better than other tissue culture materials, and it provides new approaches to understanding and controlling the behavior of cells in three-dimensional settings, a major issue in the burgeoning field of tissue engineering.

Cukierman E, Pankov R, Stevens DR, Yamada KM: Taking cell-matrix adhesions to the third dimension. Science 294: 1708-1712, 2001.

## New Taste Receptor Identified

*Background:* Taste exits in four basic flavors: sweet, sour, bitter, and salty. Though each of these four sensations is familiar to nearly all people, little is known about the specific molecular components that process and transmit taste to the brain. Several distinct taste receptors have been identified in the past few years, including those for sweet and sour; however, additional receptors remain to be found on the taste buds that line the surface of the tongue. Their discovery will provide scientists with important additional information to develop a comprehensive understanding of the biology of taste.

*Advance:* NIH scientists and their collaborators recently discovered a taste receptor that binds most of the 20 naturally occurring amino acids. The scientists speculated that the amino-acid receptor might have evolved to help people select foods that are rich in these essential nutrients. According to the scientists, the amino-acid receptor is related to the previously identified sweet receptor, in that both are different structural combinations of a family of taste receptors called T1R. The scientists noted that in humans, unlike mice, the amino-acid receptor is much more specifically tuned to recognize one amino acid in particular – glutamate. Glutamate occurs naturally in certain foods, such as seafood, and is often added to modern processed food as the flavor enhancer monosodium glutamate, or MSG.

*Implications:* The amino-acid receptor has opened up a new biological pathway to explore the sensation of taste. As scientists piece together the molecular hardwiring of this pathway, it will likely lead to rational and effective approaches to stimulate the consumption of nutritious foods among those with poor appetites, such as the elderly or people with diabetes. Toward this end, the scientists have begun attempts to create laboratory mice that lack the amino-acid receptor to define the relationship between the receptor and taste perception.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS: An amino-acid taste receptor. Nature 416: 199-202, 2002.

## Genome of Oral Pathogen Fully Sequenced

*Background:* Of the over 500 microorganisms known to inhabit the human mouth, none has attracted more attention than *Streptococcus mutans*. Decades of research has established that this bacterium is among the first microorganisms to colonize dental enamel, serving as a platform for other organisms to adhere to our teeth. Numerous studies have also implicated *S. mutans* as directly contributing to tooth decay.

*Advance:* A team of NIH grantees has finished recording the complete DNA sequence of *S. mutans*. The bacterium's genome consists of just over 2 million bases, or units, of DNA and is comprised of 1,963 recognized genes. Of the latter total, scientists already have ascribed likely biological functions to 63 percent of the genes, an important head start in piecing together the protein pathways that power this organism. The genes are involved in a range of biological tasks including cell division, cell wall synthesis, metabolism, transport, virulence, adherence, signaling, and other functions.

*Implications:* Because the *S. mutans* sequence database is freely accessible online, it will serve as an invaluable research tool to the research community. Scientists throughout the world will be able to access the database from their offices, scroll through the tables of information, form hypotheses, and rapidly test their theories in the laboratory. This genetic blueprint will trigger only accelerate investigation into the cause of tooth decay, it would suggest points of weakness in the bacterium's life cycle that could be exploited to prevent or control the formation of dental plaque, the source of caries and gum disease. They will discover in weeks information that once took decades or entire careers to obtain.

Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, Primeaux C, Tian R, Kenton S, Jia H, Lin S, Qian Y, Li S, Zhy H, Najar F, Lai H, White J, Roe BA, Ferretti JJ: Genome Sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. PNAS 99(22): 14434-14439, 2002.

## Gene Identified as Causing Inherited Gingival Overgrowth

*Background:* Dental researchers have known for decades that people with the rare hereditary gingival fibromatosis are born with gums (gingiva) that, to varying degrees, overgrow the teeth. What they have never known is why this chronic overgrowth occurs. Finding an answer will be important not only for people with HGF, but for thousands of Americans who develop gingival overgrowth. Included among this group are people born with several human syndromes and those who develop the problem as a side effect of certain frequently prescribed medications. Researchers estimate gingival overgrowth affects about 15 percent of patients who use phenytoin for seizures, about 15 percent of those who take calcium channel blockers for hypertension, and approximately 30 percent of individuals who take cyclosporine for autoimmune disease. About 40 percent of organ transplant patients who combine cyclosporine and the calcium channel blocker nifedipine, have gingival overgrowth.

*Advance:* NIH grantees and their collaborators identified the first gene that, when inherited in an altered form, triggers the most common form of hereditary gingival fibramotosis Type I, or HGF1. The gene, called SOS1, encodes a protein that is known to activate the *ras* pathway, one of the key growth signals in our cells.

*Implications:* This finding suggests that, when the SOS1 gene is not mutated its protein and the ras pathway are likely involved in the normal growth of healthy gums, an idea that was previously unknown. If confirmed, learning how to turn on relevant portions of the pathway, like flipping a biological switch, might help dentists one day regenerate the gingiva naturally in people with receding gums or advanced periodontal diseases. Conversely, by switching off the growth signal, dentists could prevent gingival overgrowth, meaning people with the condition might not need to have the excess tissue surgically cut away, now the standard treatment.

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## Advance in Understanding Biology of Pain

*Background:* When someone cuts their finger, blood and immune cells immediately release serotonin [5-HT], a neurotransmitter. The serotonin molecules rapidly bind with a number of nearby sensory receptors, which receive and process signals of tissue damage. Among them is 5-HT3R, a protein receptor found throughout the central and peripheral nervous system. Though 5-HT3R clearly plays a critical role in this so-called "nociceptive" response, questions remain about how the receptor processes this information and relays it to the brain in the form of pain. A more detailed characterization of 5-HT3R would have important implications in developing new methods to control acute and chronic pain.

*Advance:* NIH grantees created mice with inactivated copies of the 5-HT3R gene, providing a unique opportunity to monitor the nociceptive response minus the receptor. After assessing responses to a standard set of sensory stimuli, the researchers concluded that the mice exhibited significantly reduced persistent pain behavior without a corresponding reduction in the inflammatory response to a tissue injury, an aspect of the nociceptive process. Interestingly, the mice showed completely normal responses to acute pain. Based on subsequent anatomical and electrophysiological evaluations, the group determined that 5-HT3R may activate specific subsets of nociceptors – the myelinated □ and a previously undefined subset of unmyelinated C-fibers--to contribute to specific features of the pain involved in tissue injury. Significantly, their data also indicated that 5-HT3R is not expressed, as previously believed, on the more predominant capsaicin-sensitive C-fiber nociceptor.

*Implications:* These results support earlier suggestions that analgesic drugs targeted against 5-HT3R cannot, by themselves, offer satisfactory pain relief. Because only a subset of C-fiber nociceptors express the receptor, most will not respond to 5-HT3R antagonists. The scientists suggest an alternative – and likely more satisfactory approach – would be to combine 5-HT3R antagonists with drugs that also interfere with the transmission of sensory signals conveyed by unmyelinated nerve fibers.

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## Exploring the Promise of Embryonic Stem Cells

Stem cells have captured the imagination of scientists and the public. With their dual capability to replicate themselves and generate daughter cells that can mature into other cell types, stem cells hold the potential to replace missing or damaged cells in a wide variety of human disorders, including Parkinson's disease, heart failure, and some insulin-dependent forms of type 1 diabetes mellitus. However, before these potential uses are attained, much basic research must be done to define the microenvironments and factors required to generate specific functional cell types.

One of the most promising lines of research has focused on embryonic stem (ES) cells, which are formed soon after an egg is fertilized and starts dividing. In the developing embryo, ES cells transform, or differentiate, into the many specialized cells that make up an organism. Research on ES cells was initially hampered by difficulties in maintaining the undifferentiated cells in culture. A major breakthrough came in 1981, when scientists succeeded in culturing mouse ES cells. Subsequent research throughout the 1980s and early 1990s, much of which was NIH-funded, showed that mouse ES cells could differentiate into heart cells, neural cells, and other cells in the body.

Unfortunately, the intricate, multistep technique developed for culturing mouse cells proved unworkable when applied to other mammalian species. Researchers quickly learned that embryos must be removed from the animal at just the right time, and embryonic cells must be cultured under specific conditions to allow them to replicate but not differentiate.

Not until 1995 did scientists achieve the right combination of techniques and conditions for producing ES cell cultures from another mammalian species. Working at the NIH-funded Wisconsin National Primate Research Center (NPRC), Dr. James Thomson and his colleagues found that they could grow ES cells from rhesus macaque embryos on a blanket of mouse connective tissue cells called fibroblasts. These "feeder cells," which secreted substances that supported the growth of the ES cells and prevented them from differentiating, proved the critical factor in producing rhesus ES cell cultures. A year later, the Wisconsin researchers succeeded in producing ES cell lines from another nonhuman primate species, the common marmoset.

Importantly, cultured rhesus ES cells appeared to be capable of differentiating into the various cell types of the body. When treated with certain chemicals in culture or injected into immunodeficient mice to avoid problems with immune rejection, the ES cells differentiated into cells found in bone, muscle, nervous system, and other tissues.

Using knowledge gained from their studies of nonhuman primate cells, Dr. Thomson and his colleagues took a significant step forward in 1998, when they isolated and propagated ES cells from humans. As in their nonhuman primate studies, the researchers derived their cell lines from cells formed only days after the egg was fertilized and started dividing. Shortly after the Wisconsin group announced their accomplishment, Dr. John Gearhart and his colleagues at Johns Hopkins University reported that they had also derived human ES cell lines, although

these researchers used as their source primordial germ cells – precursors of human sperm and eggs – taken from medically aborted 5- to 8-week-old embryos. Both lines of research were conducted independently of NIH support and together set the stage for exploring the use of human ES cells for cell replacement therapies. Subsequently, other research groups developed their own human ES cell lines.

Now that ES cell lines from multiple species are available, scientists are working with animal models to determine whether stem cell transplants can treat human diseases. One area of intensive research involves transplanting stem cell derivatives into the brain or spinal cord to treat neurological disorders or injuries. Dr. Thomson and his colleagues recently derived neural precursor cells from cultures of human ES cells and transplanted the precursors into the brains of neonatal mice, where they formed nerve cells, or neurons, and support cells called astrocytes.

Other research shows that cells derived from ES cells can be functional. Recently, scientists at Harvard University injected mouse ES cells into the brains of rats with a condition similar to Parkinson's disease. In this disease, some of the neurons that produce the messenger chemical dopamine have degenerated, causing abnormal movements. The scientists found that the transplanted ES cells differentiated into the dopamine-producing neurons, thereby allowing the rats to perform normally in a motor test. The Harvard team is now collaborating with researchers at the Wisconsin NPRC to perform similar experiments with rhesus monkeys.

Using animal models of diabetes, in which the insulin-producing cells in the pancreas have been destroyed, researchers are also exploring the possibility that transplanted stem cells might differentiate into insulin-producing cells and reverse the diabetes. Spanish researchers have transplanted insulin-producing cells derived from mouse ES cells into the spleens of diabetic mice, where they secreted enough insulin to normalize blood glucose levels. Scientists at the Oregon NPRC are now attempting to derive insulin-producing cells from rhesus ES cells.

Although investigations of human ES cells were initially funded via non-Federal sources, the U.S. government in 2001 allowed the use of Federal funds for studies with approved, pre-existing human ES cell lines, including several derived by Dr. Thomson's laboratory. Recently, NIH gave a major boost to the field of human ES cell research by providing four resource infrastructure enhancement awards for the characterization, scale-up, and distribution of 17 of the human ES cell lines. Besides distributing the cells, grantees will also perform quality control tests, test for pathogenic organisms, and derive sublines with useful characteristics, such as the expression of cell markers. In addition, they will train investigators to successfully establish human ES cell cultures in their laboratories.

To facilitate research on animal stem cells, NIH has also established the National Stem Cell Resource, which will provide embryonic and postnatally derived stem cells from a variety of nonhuman species. The resource will standardize culture methods and develop new reagents related to stem cell characterization and utilization.

Besides studying the possible use of human ES cells to replace damaged tissues, scientists are also investigating the possibility that so-called adult stem cells may work as well as ES cells. As the name suggests, adult stem cells are formed later in development and remain undifferentiated long after birth. While ES cells are capable of generating any cell type, adult stem cells generally differentiate into only a few cell types. Lately, however, evidence suggests that adult stem cells may be more flexible than previously thought, although additional research is needed.

The apparent versatility of embryonic and adult stem cells makes them likely candidates for replacing many current drug and medical interventions. Instead of treating Parkinson's patients with the drug L-DOPA, which eventually loses its effectiveness, patients might receive stem cell transplants in the brain, where the cells might form the missing dopamine-producing neurons. Instead of treating diabetes with insulin injections, patients might receive stem cell transplants that generate needed insulin-producing cells. Stem cell transplants might also be used to treat diseases for which effective treatments currently do not exist, such as Alzheimer's disease.

Many scientists view the culturing of human stem cells as an unprecedented scientific breakthrough. With their potential for generating almost every cell type in the human body, stem cells may eventually revolutionize the practice of medicine and improve the quality and length of life.

## Alcoholic Liver Disease

Alcohol-induced liver disease (ALD) is a major cause of illness and death in the United States, affecting an estimated 2 million people in this country. A result of long-term alcohol use, ALD begins with a buildup of fat in the liver and in about one-third of heavy drinkers progresses to the persistent liver inflammation known as alcoholic hepatitis. Cirrhosis, a progressive scarring of the liver, is the final stage of ALD and develops in 10 to 20 per cent of heavy drinkers.

Abstinence from drinking is the primary therapy for ALD and can reverse many cases of fatty liver and alcoholic hepatitis. But even highly motivated people with alcoholism and other drinking problems can find it difficult to stop drinking. Damage from cirrhosis is irreversible and often requires liver transplantation, a costly, high-risk procedure.

Scientists supported by NIH continue to investigate how alcohol interacts with the body to cause ALD. Knowledge gained through these studies could lead to new therapies to prevent some of the medical complications of ALD or reduce the severity of the illness. Over the past few years, NIH-supported scientists led by Dr. Ron Thurman at the University of North Carolina have used gene "knockout" techniques to shed important new light on the molecular mechanisms that underlie ALD. Gene knockout technology allows scientists to develop strains of laboratory animals that lack a specific gene or genes. Gene knockout animals therefore lack the proteins encoded by the missing genes. Through experiments that compare gene knockout animals with normal animals, scientists can definitively determine what role, if any, a particular gene plays in disease.

For some time, scientists have known that intestinal bacteria play a key role in ALD. Researchers have prevented ALD in laboratory animals by eliminating normal intestinal bacteria with antibiotics or through dietary manipulation. A bacterial component known as lipopolysaccharide (LPS), or endotoxin, is the chief cause of the problem. Long-term drinking makes the intestines leaky, allowing LPS to pass into the bloodstream.

Specialized liver cells known as Kupffer cells trigger an inflammatory response when they detect LPS in the blood. The prolonged liver inflammation seen with long-term alcohol use can damage or kill liver cells. Kupffer cells are directly involved in alcohol-induced liver injury – in 1994, Dr. Thurman's group showed that ALD symptoms in rats can be prevented by a chemical that selectively kills Kupffer cells. More recently, they have found that several proteins involved in Kupffer cells' response to LPS are major culprits in alcohol-induced liver injury.

LPS becomes attached to Kupffer cells with the help of a binding protein known as lipopolysaccharide binding protein, or LBP. Dr. Thurman's group compared the effects of long-term alcohol intake in two groups of mice - those with and those without the LBP gene. For four weeks, the mice received an alcohol-rich diet through a feeding tube attached to their stomachs. In normal mice, chronic alcohol intake produced ALD symptoms such as fatty liver,

inflammation, and liver tissue damage. These symptoms were significantly less pronounced, however, in the LBP gene knockout mice that had received the same high-alcohol diet.

The molecules known as CD14 and TLR4 on Kupffer cells play essential roles in generating the liver's inflammatory response. Research evidence suggests that CD14, which lies on the surface of the Kupffer cell, initiates the inflammatory signal when it interacts with LPS. TLR4 lies within the Kupffer cell membrane, where scientists believe it acts as an intermediary, relaying the message farther down the signaling pathway.

Since inflammation is known to contribute to liver damage, Dr. Thurman's group assessed whether CD14 and TLR4 play a direct role in alcohol-induced liver injury. In one experiment, normal mice and CD14 knockout mice were maintained on the same high-alcohol feeding regimen used in the LBP experiments. The alcohol diet induced severe liver injury in the normal mice. It also increased the production of tumor necrosis factor, or TNF, one of a class of molecules known as cytokines that are released as part of the normal inflammatory response. Excess production of TNF and other cytokines can damage liver cells. The CD14 knockout mice, on the other hand, experienced only minimal liver damage and no increase in TNF, confirming that the CD14 receptor is necessary for alcohol-induced liver injury.

In another experiment, Dr. Thurman and his colleagues administered the alcohol diet to normal mice and to mice with TLR4 gene mutations. After 4 weeks, normal mice had fatty liver, liver inflammation and tissue damage, and other signs of liver injury. The TLR4 mutant mice, however, had significantly less liver injury than the normal mice did, indicating that TLR4 also is necessary for alcohol-induced liver injury. Taken together, these results suggest that heavy alcohol use increases bloodstream levels of LPS. Excessive LPS then stimulates the Kupffer cell's LBP-CD14-LTR4 inflammation-signaling pathway to cause ALD. Compounds that disrupt Kupffer cell inflammation signaling may be useful in preventing or treating alcoholic liver injury.

In addition to LPS, compounds known as reactive oxygen species, or oxidants, are known accomplices in alcoholic liver disease. Oxidants are toxic molecules that are produced throughout the body during a wide variety of physiologic processes. Normally, oxidants are quickly inactivated by antioxidants such as vitamins A and E. Long-term heavy drinking, however, leads to overproduction of oxidants, which can overwhelm the body's natural antioxidant defenses and lead to destruction of liver cells. At least two separate enzymes produce oxidants when the liver interacts with alcohol. A liver enzyme known as CYP2E1 produces oxidants as it breaks down alcohol. A Kupffer cell enzyme known as NADPH oxidase, which is revved up when Kupffer cells detect LPS in the blood, also generates oxidants.

The Thurman group used gene knockout techniques to evaluate the contribution that each oxidant-generating mechanism makes to alcohol-induced liver disease. First, they compared the effects of the 4-week, high-alcohol diet in normal mice and in CYP2E1 knockout mice. Both groups of mice experienced the same degree of liver injury. If CYP2E1 was a factor in alcohol-

induced liver injury in the mouse, then the CYP2E1 knockout mice would have been protected. Instead, the results indicate that CYP2E1-generated oxidants are not involved in the mouse model of ALD. Conversely, NADPH oxidase knockout mice experienced no adverse effects from chronic alcohol intake, suggesting that oxidants generated by Kupffer cells play an important role in alcohol-induced liver injury.

Tumor necrosis factor also has been suspected of contributing to ALD, given its known toxicity and increased production by Kupffer cells in response to LPS. TNF exerts its toxic effects by interacting with specific receptors. To determine if TNF participates in alcohol-induced liver injury, researchers compared the effects of the alcohol diet in three groups of mice: normals, and those in which the TNF R1 or TNF R2 receptors had been knocked out. The normal mice and the TNF R2 knockout mice developed liver damage. The TNF R1 knockout mice, however, were unharmed by long-term alcohol exposure, indicating that the R1 receptor must be present for alcohol to induce liver damage.

In response to TNF production, cells on the surface of the liver begin to produce intercellular adhesion molecules, or ICAMs, which attract inflammatory cells and help them penetrate the liver. Inside the liver, these cells release oxidants and other inflammation-producing compounds that can damage or destroy liver cells. Scientists suspected that the ICAMs might therefore play a role in alcohol-induced liver injury. Dr. Thurman's group confirmed these suspicions by showing that ICAM knockout mice were protected from the severe liver damage that normal mice experience from long-term alcohol intake.

Taken together, this series of gene knockout experiments has given scientists a much clearer picture of the cellular and molecular mechanisms of alcohol-induced liver injury. LBP helps LPS bind to Kupffer cells, where LPS interacts with CD14 to initiate an inflammatory signal. The signal is relayed to LTR4 within the Kupffer cell membrane. The CD14 and TLR4 molecules stimulate TNF production, which, in turn, induces the expression of ICAMs on liver cells. Inflammatory cells recruited by ICAMs then bombard liver cells with oxidants and other damaging compounds. At the same time, Kupffer cells generate oxidants in response to bloodstream LPS. This complex pathway provides a number of possible targets at which new therapies might be directed.

## Harnessing Apoptosis to Destroy Cancer Cells

In 1972, John Kerr, Andrew Wyllie, and Alistair Currie published a paper describing a little known and curious form of cell death that today is one of the most intensively studied topics in modern biology. The researchers reported on a type of cell death – a programmed cell suicide – that was distinctly different from the long-recognized process of cell death known as necrosis. Necrosis occurs when a cell becomes acutely injured and ruptures, causing inflammatory cells to rush in to clear away the debris. Programmed cell suicide, in contrast, is clean and quick and involves a predictable sequence of structural changes that cause a cell to shrink and be rapidly digested by neighboring cells. Although biologists have long known that cell suicide plays an important role in sculpting tissue within developing embryos, Kerr, Wyllie, and Currie were the first to observe that programmed suicide – which they labeled apoptosis – also occurs in mature cells. They also were the first to hypothesize that apoptosis plays a broad role in normal life processes, and its failure – which disrupts the balance of cell death and renewal – contributes to a variety of diseases, including cancer.

Unfortunately, this groundbreaking paper created little excitement in the scientific community until more than ten years later when H. Robert Horvitz used the microscopic roundworm *C. elegans* to explore how a single fertilized egg develops into an adult organism with multiple cell types. As he painstakingly followed each of the developing worm's 1,090 cells to their ultimate fate, he was surprised to see that 131 cells died via apoptosis as the worm matured into adulthood. With this observation, he substantiated the prediction made by Kerr and his colleagues that apoptosis occurred beyond embryogenesis. By1986, Horvitz determined that three genes – *ced-3*, *ced-4*, and *ced-9* – were responsible for regulating apoptosis in *C. elegans,* demonstrating for the first time that programmed cell suicide is genetically programmed. The *ced-3* and *ced-4* genes produce proteins that initiate apoptosis; the *ced-9* gene generates a protein that inhibits it. Horvitz and his colleagues also determined that these genes are broadly conserved among plants and animals, indicating that this process has been sustained through evolution and has a universally important biological function.

The Horvitz findings greatly energized apoptosis research. Over the next 15 years, using developing technologies and tools, scientists demonstrated that apoptosis helps to establish a natural balance between cell death and cell renewal in mature plants and animals by destroying excess, damaged, or abnormal cells. Further exploration revealed that apoptosis occurs through two distinct cellular pathways, one which is initiated from outside of the cell, the other from within. Both pathways were shown to converge inside the cell, somehow turning on a central executioner family of proteins known as caspases. Caspases act as knives, cutting up proteins inside the cell and digesting the cell from within. Scientists realized that finding the trigger of caspase activity would offer the unprecedented opportunity to control cell death and survival. After several years, in 1996, Xiaodong Wang and colleagues discovered that caspases are activated by cytochrome c, a critical protein component of the mitochondria (the energy-producing structures of cells). With this finding, scientists began to study the mitochondria to determine how apoptosis functions in the cell, and malfunctions in disease.

*Connecting Failed Apoptosis and Cancer*. Kerr and colleagues had speculated – and subsequent studies hinted – that inappropriate apoptosis activity could contribute to a number of diseases. However, the link between apoptosis and cancer was not established until 1988 when David Hockenbery and colleagues characterized the *bcl-2* gene. *Bcl-2* was first discovered in B cells (an immune cell) from patients with follicular lymphoma (an immune system cancer). Hockenbery determined that the normal *bcl-2* is a suicide "brake" gene – it produces a protein that blocks apoptosis. In lymphoma patients, however, the abnormal form of the gene is overactive, causing the anti-apoptosis protein to be overproduced. Cancer develops as more and more B cells are generated and fail to die. This finding, considered to be a milestone in cancer research, revealed that increased cell division was not the only way that tumors could develop. Cells could also become potent promoters of tumor growth by avoiding programmed cell death.

Throughout the 1990s, scientists gathered considerable information about bcl-2. They determined that increased bcl-2 protein production occurs in several cancers (B cell leukemias, lymphomas, colon and prostate cancers, and neuroblastoma) and is linked with poor disease outcome. In addition, overexpression of the *bcl-2* gene may confer resistance to chemotherapeutic drugs. Yet, it was not until 1997 that scientists determined how the bcl-2 protein controls apoptosis: bcl-2 blocks the release of the caspase trigger – cytochrome c – from inside the mitochondria. Scientists now are working to develop a complete picture of how bcl-2 controls cytochrome c release so that they can improve the suicide-provoking effects of cancer treatments as well as thwart a cancer cell's ability to evade these drugs.

Although bcl-2 was the first component of the cell suicide mechanism to be identified, scientists have determined that this dauntingly complicated process has many genetic controls. For example, the p53 protein, known as the guardian of the human genome, acts to suppress tumor formation partly by killing off cells with damaged DNA. Upon detecting the damage, p53 either temporarily arrests the cell cycle to allow repair or triggers apoptosis by attacking the mitochondria, making it release its cytochrome c. In 55 to 70 percent of human cancers, however, genetic mutations render the p53 protein deficient and cells with DNA damage can continue to accumulate. Loss of p53 function is associated with tumor aggressiveness and resistance to anti-cancer treatments.

Mounting evidence indicates that the acquired ability to resist apoptosis is a hallmark of most, and perhaps all types of cancer. As scientists learn more about how apoptosis fails in cancer, they also are gaining a greater understanding of why many tumors are resistant to the killing effects of radiation and chemotherapy, which both act by inducing cell suicide. These insights can inform efforts to overcome treatment resistance and offer important clues about new drugs that target genes and protein products in the apoptosis pathways to encourage selective cell death. Researchers are now exploring how apoptosis is regulated, how it might be repaired through genetic therapies, and how it can be selectively triggered, through tailored treatments, to induce suicide in cancer cells while leaving healthy cells alone.

*Triggering Apoptosis with New Cancer Drugs*. Clinical trials are currently underway to test the efficacy of new apoptosis-inducing drugs. Velcade, a new agent jointly developed by NIH and Millenium Pharmaceuticals, targets the proteosome, a device inside a cell that functions like a cellular "garbage disposal," removing abnormal, aged, or damaged proteins. By blocking the activity of the proteosome, Velcade causes proteins, including those that induce apoptosis, to build-up in the cell. Velcade may prove to be a versatile cancer treatment because it appears to be equally effective against cancers that do or do not overexpress the *bcl-2* gene and seems to overcome a tumors ability to develop chemoresistance. In a Phase II clinical trial of patients with progressing multiple myeloma, Velcade stabilized the disease in 77 percent of the trial participants. Based on this encouraging result, researchers are planning a Phase III trial to compare Velcade to dexamethasone, a chemotherapy now used to treat multiple myeloma. Other Phase II trials will determine the drug's effectiveness in treating breast cancer, non-small cell lung cancer, melanoma, sarcoma, chronic myelogenous leukemia, non-Hodgkin's lymphoma, and neuroendocrine and renal cancers.

Genasense is another apoptosis-inducing agent that is being tested for its clinical use. Developed by the Genta Company, this drug blocks the production of the bcl-2 protein and leaves cancer cells more vulnerable to apoptosis-inducing chemotherapies. NIH and Genta are cosponsoring clinical trials in lung cancer and leukemia patients to determine whether pretreatment with this drug followed by state-of-the-art chemotherapies improves treatment outcome.

## Lysosomal Storage Diseases

The lysosomal storage diseases (LSDs) defied the best efforts of medical science since physicians first recognized these inherited disorders in the 19th century. However, persistent research focused on the LSDs, coupled with advances arising from fundamental biology, are bringing progress in understanding, diagnosing, preventing, and treating these diseases.

We now group more than 40 disorders as LSDs. Each occurs when storage material accumulates in a recycling compartment of cells called the lysosome. The material accumulates because an inherited defective enzyme – a different enzyme in each disease – fails to break down certain complex biological molecules. The accumulating materials damage cells, leading to a host of problems that can involve the brain, heart, kidneys, spleen, the skeletal system, and the eye. In the most serious cases, an infant at first develops normally, then progressively loses even the most basic abilities and dies. In other cases symptoms may be quite mild, first appearing in adulthood. Even a single disorder can produce a wide range of symptoms and severity in different people, further complicating the picture for these diseases.

In the 1920s and 1930s, researchers, relying upon advances in fundamental biochemical science, began to make inroads into understanding the LSDs by identifying the complex lipids (fatty chemicals) that were accumulating in the brain and other organs. Lipids provide electrically insulating covering for nerve fibers, are a crucial component of cell membranes, and serve many other normal functions. Why these lipids were building up in LSDs was a mystery. Since the 1950s, Dr. Roscoe Brady, in the NINDS intramural research program, has led a pioneering research team that has improved the understanding, diagnosis, and treatment of LSDs. Building on knowledge from studies of the normal metabolism of lipids, the researchers determined that patients with Gaucher disease, an LSD, accumulate the lipid glucocerebroside because they inherit a defect in the enzyme glucocerebrosidase. The critical studies used newly emerging technology for tagging chemicals in the body with trace radioactive substances to follow metabolism.

Having identified defective glucocerebrosidase as the culprit in Gaucher disease, replacing the missing enzyme seemed a logical approach to therapy. However, it took eight years to develop methods to purify enough glucocerbrosidase from human placental tissue to attempt treatment in just two patients. The results were encouraging. The NINDS research team, working with a private biotechnology company, went on to develop a method that not only produced sufficient quantities of glucocerebrosidase, but also modified the enzyme so that cells were more likely to take it up where it was needed. Clinical trials demonstrated that enzyme replacement therapy dramatically improves quality of life for people with Gaucher disease.

Following the pattern for attacking Gaucher disease, Dr. Brady's team subsequently identified the specific enzyme defects in Niemann-Pick, Tay-Sachs, and Fabry diseases. They and other scientists have now determined the defects in more than 40 LSDs. For some of these disorders, enzyme therapy is also proving effective. For example, last year an NIH clinical trial

demonstrated that enzyme therapy for Fabry disease provides widespread benefit for patients with the disorder. The enzyme reduced the level of severe pain, improved pain-related quality of life, and appeared to reduce kidney problems and improve cardiac function. Trials for enzyme replacement therapy in other LSDs, such as the mucopolysaccharidoses, are underway here and abroad.

Enzyme therapy for Gaucher and Fabry diseases is a remarkable achievement. It has saved or dramatically improved thousands of lives and demonstrated the feasibility of this approach for several other diseases. However, it is not the final answer for the LSDs. Enzyme therapy is extremely expensive and, more importantly, it cannot address the brain dysfunction caused by LSDs. The blood-brain barrier, which protects sensitive brain cells from potentially toxic chemicals in the general circulation, also prevents large molecules such as enzymes from reaching brain cells. Researchers are developing other strategies for treatment. One approach currently under testing capitalizes on detailed understanding of the biochemical pathways by which cells make lipids. Scientists have developed drugs to reduce the synthesis of lipids that lead to storage products in the brain in LSDs, thereby minimizing the accumulation of harmful substances. Following promising findings in animal models of LSDs, this therapeutic strategy is now being tested in patients.

The advent of the molecular biology era has opened another approach for attacking these diseases. Various research teams here and around the world have identified the specific gene defects responsible for most of the other LSDs. This has enabled scientists to develop tests that improve diagnosis and carrier screening. Most LSDs are recessively inherited. A child must inherit a defective gene from both parents to acquire the disease, and carriers of one gene usually show no overt signs of the disorder. Since some of these diseases are concentrated in specific population groups, carrier screening can be crucial. For some diseases, such as Tay-Sachs, screening, with genetic counseling, has dramatically reduced the number of new cases.

The advances in molecular genetics have also offered the tantalizing hope of gene therapy to replace or repair the defects that cause LSDs. Gene therapy is a deceptively simple idea which is extraordinarily difficult to carry out safely and effectively. For the LSDs this is particularly so because the gene must be supplied to cells throughout the body, including the brain. The brain is an especially difficult target for gene therapy, not only because of the blood-brain barrier, but also because nerve cells do not divide and thus are not amenable to the most common strategies to introduce genes. Nonetheless, there has been encouraging progress toward developing gene therapy for LSDs. Relying upon natural mutations and genetic engineering, scientists have developed strains of mice that mimic several human LSDs. These mice provide models for testing potential approaches to gene therapy. Dr. Brady's group provided the first successful safety test for this approach in 1995, and efforts by several research teams are now underway.

Gaucher disease was first reported in the medical literature in 1882. By the 1920s, biochemical science had advanced sufficiently to identify the chemical that accumulates in this disorder, but it wasn't until the 1950s, with advances in radionuclide chemistry, that scientists could begin to

determine just why this was occurring. It took five years to discover the enzyme defect, eight more years to purify enough enzyme for the first human tests, and sixteen years more to produce enough enzyme to treat many patients and to establish the striking effectiveness of enzyme replacement therapy.

## How The Brain Tells The Eye Where To Go

One type of movement, called a saccade, rapidly points the eye at an object of interest. How the brain generates the nerve impulses needed to move the eye during a saccade has been a matter of intense investigation for over 30 years. Historically, models of the neural control of movement have been based on classical systems or control theory principles. An example of this approach is represented by the target-tracking model of the saccadic system proposed over 25 years ago. Such models have helped us formalize the problems that the brain faces, provided insights into adaptive processes, and often inspired further experiments. However, control system models do not accurately represent brain structure and activation patterns. This makes it difficult or impossible to explain eye movement deficits resulting from injury or disease, or to predict the effects of novel experiments. Worse, they can impede the achievement of a thorough understanding of the brain by forcing our interpretations of experimental data to match arbitrary expectations.

For these reasons, it is important to develop models that mimic actual brain structure and neuronal activity. One of the key advantages of these new models, which are called neuromimetic, is their ability to predict behavior under novel experimental conditions. Furthermore, they can provide insight into the nature of neural signals and their encoding, at both the single neuron and population level. Recently, scientists proposed a neuromimetic model of the saccadic pulse generator that makes realistic movements and accurately reproduces neuronal activity under different experimental conditions; yet it does not fit into any classical controller scheme. This lack of conformance led to an investigation of whether some general principles about information processing during the neural control of movement could be inferred from the neuromimetic model.

The major difference between a classical and a neuromimetic model of the saccadic system is in the way they encode signals. Classical models represent physical signals, such as the desired change in eye orientation or the motor error (the difference between where the eye is and where it wants to go ), as internal variables. In contrast, a neuromimetic model uses spatial maps, wherein the location of a cell in the brain lets it represent information. Connections between these maps allow the brain to process signals that are not related directly to physical signals. An example of where this is important is how the brain tells the eye where to go. In the neuromimetic model, two areas, one in the brain stem and one in the cerebellum, both contain spatial maps encoding information needed to specify the desired eye movement. The brain stem map represents which target should be looked at, in visual coordinates, and the cerebellar map represents what is known about the context of the movement, such as the starting position of the eyes. It takes the output of both of these areas to determine where the eye will end up. Thus, there is no single area or signal encoding the desired eye displacement.

Comparison of classical and neuromimetic models suggests that the encoding of movement signals in the brain may occur in a completely unorthodox way, one that does not internalize the physical signals (e.g., motor error or desired displacement) associated with the movement.

Instead, intrinsic brain signals may represent desired sensory states (i.e., which target needs to fall on the fovea after the movement), approximate motor drives, and distributed motor commands. The neuromimetic model is based on what is known about the physiology and anatomy of the saccadic system. It demonstrates that, through the proper connection of many computationally simple elements, nature can devise solutions to control problems that are unorthodox, yet have advantages over classical control schemes. This new class of neuromimetic models also provides advantages for understanding eye movement deficits that are the result of disease, trauma, or inheritance, because they directly represent activity of brain cells in several areas.

## The Search for the Master Reproductive Hormone

In 1977, NIH grantees Andrew Schally and Roger Guillemin jointly received the Nobel Prize in Medicine for independently discovering the hormones produced by the brain structure known as the hypothalamus. Their discovery led to the eventual development of treatments for prostate cancer and female infertility. Moreover, their work provided the theoretical framework for new areas of research that offer the hope of even better treatments for these conditions, as well as for a male contraceptive.

Our understanding of the exact role played by the hypothalamus has evolved through a series of scientific discoveries, spanning more than 80 years. The hypothalamus is the brain region that regulates important bodily functions, such as blood pressure, body temperature, fluid balance, and body weight. The hypothalamus accomplishes many of its functions by releasing hormones that send signals through a network of blood vessels to the pituitary. The pituitary is a pea-sized gland that lies beneath the brain and is the central control point for growth, maturation, and reproduction.

Attempts to learn the purpose of the hypothalamus began in the 1920s, when scientists cut the blood vessels leading from the hypothalamus in laboratory animals. The researchers observed that when these connections were severed, animals lost their reproductive functioning. This led researchers to search for a "master" reproductive hormone, which they called luteinizing hormone releasing factor (LHRF). LHRF, they theorized, signaled the pituitary to release luteinizing hormone. Scientists knew that luteinizing hormone acted on the ovaries and testes, eventually leading to ovulation in women and sperm production in men.

Later, two groups of NIH-supported scientists, one led by Schally, the other by Guillemin, joined the search for the elusive hormone. After a close competition to be first, the two groups independently found the hormone. Guilleman’s group found the mysterious substance by looking in a million portions of discarded sheep brains. Schally’s group reported finding an identical substance from an equal amount of pig brain tissue. Since they lacked today's precision techniques for isolating a minute substance, the researchers needed massive quantities of tissue to work with. The hormone produced by the hypothalamus is now known as gonadotropin releasing hormone (GnRH). GnRH was later found in a number of species, including human beings. This discovery opened the door for a series of subsequent advances in treating reproductive health abnormalities and cancers of the reproductive system.

Researchers began to develop GnRH analogs – drugs that were chemically very similar to GnRH, but, due to minor substitutions in their chemical makeup, could have similar or opposite metabolic actions. For example, a research team found that injecting lab animals with a large dose of GnRH analogs could over-stimulate their reproductive systems. The animals' systems would at first respond with a surge of reproductive hormones, but eventually stop producing the hormones completely.

Following up on this discovery, a pharmaceutical company developed another analog, leuprolide, which later became a routine treatment for prostate cancer in men. Often, prostate cancer cells require the male hormone testosterone. Taking the drug initially causes an increase in testosterone, but eventually causes the body to stop producing it completely. This deprives the tumors of the testosterone they need, causing them to grow more slowly. Leuprolide can also be used as a treatment for precocious puberty, a condition in which boys and girls begin the process of sexual maturation abnormally early. Physicians prescribe leuprolide to switch off sex hormone production until a child reaches the appropriate age. This is the first effective treatment for precocious puberty and is now standard therapy for this condition.

Other analogs have been used to treat female infertility. Essentially, physicians use these drugs to "reset" a woman's monthly ovulatory cycle so that they can precisely time ovulation. The GnRH analogs stop a woman's cycle completely – other hormones are then given to bring about ovulation.

Recently, other NIH-funded researchers developed a new GnRH analog known as acyline. Acyline causes the body to stop producing sex hormones immediately and does not cause the painful swelling at the injection site associated with earlier analogs. Preliminary studies have shown that the compound is safe as well as effective at shutting down the reproductive cycle. Researchers also hope to use acyline or other new GnRH analogs as part of a male contraceptive. These new analogs would cause the testes to stop producing sperm. However, since their bodies' would stop producing testosterone, men using these analogs would also lose their sex drive. NIH scientists are now working to refine the drug to make it easier to take and to reduce the undesirable side effects.

Although scientists have made tremendous progress in understanding the function of GnRH, future research is needed to expand our knowledge about this very important hormone and to understand the potential of GnRH analogs for treating a variety of reproductive abnormalities and certain cancers of the reproductive system.

## Evolving Microbes

Deadly viruses like HIV or Ebola and menacing bacteria such as those that cause strep throat infections are true chameleons of nature. These and other disease-causing microorganisms adapt to a seemingly unlimited array of conditions by making rapid genetic changes. This microbial evolution outpaces the evolution of the human species by millions of years and creates a moving target for drug designers. By analyzing the evolution of infectious organisms, researchers now have a leg up on how to outwit potentially dangerous microbes. This understanding has helped explain why some microorganisms cause disease and some do not, and it is helping scientists develop life-saving treatments and vaccines. The predictive power of this relatively new brand of research may also aid in preventing disease outbreaks.

Over the past decade, an explosion of knowledge about the genetics of viruses, bacteria, and fungi has launched a new field that marries evolutionary biology and the study of infectious disease. Sophisticated mathematical and computer-based approaches are helping researchers weave the two areas together. Interesting recent discoveries in this field that have clear relevance to public health are highlighted below. The findings reflect advances in knowledge about the genetic gymnastics of several different microbes, including *Streptococcus pyogenes* (*S. pyogenes*, one form of strep bacteria), influenza (the flu), and *Helicobacter pylori* (*H. pylori*, the bacterium that is the leading cause of digestive tract ulcers), as well as about the serious problem of antibiotic drug resistance.[6]

***Strep –*** *S. pyogenes* is a master of infection, causing a spectrum of illnesses from strep throat and tonsillitis to scarlet fever, toxic shock syndrome, sepsis, and flesh-eating disease.[7] Humans are the only known host for this group of bacteria. Scientists believe that *S. pyogenes* can cause so many different health problems because its set of genetic instructions varies widely among strains. Subtle genetic changes in these strains permit the bacterium to thrive in a variety of body locales, such as the throat or skin. Using mathematical approaches coupled to genetic studies, biologists have pinpointed several genes that permit this microorganism to live well in either the skin or the throat, but not in both. These genes may be attractive targets for drug development.

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[6] *H.pylori* and Peptic Ulcer fact sheet, National Institute of Diabetes and Digestive and Kidney Diseases, NIH Publication No. 01-4225, October 1997 (updated at ttp://www.niddk.nih.gov/health/digest/pubs/hpylori/hpylori.htm, July 2001).

[7] Ferretti JJ, McShan WM, Ajdic D, Savic DJ, Savic G, Lyon K, Primeaux C, Sezate S, Suvorov AN, Kenton S, Lai HS, Lin SP, Qian Y, Jia HG, Najar FZ, Ren Q, Zhu H, Song L, White J, Yuan X, Clifton SW, Roe BA, McLaughlin R: Complete Genomic Sequence of an M1 Strain of *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. 98: 4658-63, 2001.

***Flu –*** The best way to fight infectious diseases is to prevent them, and a key element of prevention is the ability to accurately predict disease outbreaks. Mathematicians have joined forces with evolutionary biologists and infectious disease specialists to develop powerful ways to track the evolution of viruses such as influenza A, the constantly changing strain usually blamed for major flu epidemics[8]. A multidisciplinary team of scientists recently analyzed a computer database containing DNA sequences representing 560 samples of different flu viruses from the last 16 years and discovered patterns of genetic changes that may allow better prediction of which strains of flu will emerge in the coming season. If accurate, such predictions will lead to more effective flu vaccines, preventing misery and saving many lives each year.

***H. pylori and anthrax*** – A recent study conducted by a biologist-mathematician team uncovered important scientific knowledge about *H. pylori*. The scientists used mathematical modeling to track different genetic variants of *H. pylori* over time. After creating their model, the researchers checked its accuracy by testing it in animal experiments. The results indicate that *H. pylori*, which often lives nearly indefinitely in its host, undergoes constant evolutionary change through indiscriminate "mating" between different genetic strains, all within the infected host. The research also had another unexpected benefit: Modeling the infectious behavior of *H. pylori* provided important clues on how the deadly bacterium *Bacillus anthracis*, which causes anthrax, could be spread through the U.S. postal system. Using similar techniques to the *H. pylori* research, the same researchers mathematically simulated the outbreak of mail-borne anthrax in the fall of 2001 and concluded that all the known cases of infection could be traced back to contamination through the mail from only six original envelopes. The scientists also concluded from this mathematical model that the rapid and widespread use of antibiotics probably averted many additional potentially deadly infections from this outbreak.

***Antibiotic resistance –*** The ability of bacteria to evolve rapidly enables them to escape the effects of antibiotics designed to kill them. Antibiotic resistance is an increasing problem throughout the world. Recently, scientists using computer simulation have been able to predict genetic changes that allow bacteria to resist antibiotics. The researchers simulated microbial evolution in the laboratory by choosing certain bacterial genes and determining through experiment which of the genes are most susceptible to changes that cause resistance to commonly used antibiotics. Remarkably, the researchers found that their modeling technique matches the bacterial evolution that occurs in nature. The researchers' novel approach is likely to have practical value in enabling pharmaceutical companies to create drugs for which bacteria have no evolutionary escape route. Such an approach could also allow drug developers to know how long an antibiotic will be useful: a few months, a year, or a decade. The ability to perform such analyses during the development phase will help to prevent the failure of antibiotic medicines in real-life use.

[8] Influenza listing of the MEDLINEplus Health Information Web site at http://www.nlm.nih.gov/medlineplus/ency/article/000080.htm.

By applying the tools of basic science to the study of bacterial evolution, scientists are attacking the problem of antibiotic resistance in novel ways. The results of their research are likely to lead to new treatment and prevention strategies that will improve human health.